

A CONTRIBUTION
TO THE
MORPHOLOGY AND BIOLOGY
OF THE STENTORS

AN INAUGURAL DISSERTATION
FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY
PRESENTED TO THE
FACULTY OF THE UNIVERSITY OF CHICAGO
APRIL 15, 1893

BY
HERBERT PARLIN JOHNSON

BOSTON, U.S.A.
PUBLISHED BY GINN & COMPANY
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HERBERT P. JOHNSON.

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I. INTRODUCTION.

OCCUPYING a place about midway between the lowest and the most highly-organized of the Ciliate Infusoria, and possessing in large measure the characteristics of both free-swimming and sedentary forms, the genus *Stentor* may well serve as type of its Class. Their abundance, large size, and strongly-marked morphological characters have made the Stentors favorite subjects for study with zoölogists ; and in recent years one species, *Stentor cæruleus*, has been repeatedly used in cytological research, particularly in experimental work undertaken to ascertain the physiological rôle of the nucleus.

In the present paper observations upon five species of American Stentors are recorded. All have been obtained in abundance except *Stentor roeselii*, which, not being gregarious, cannot be collected in large numbers.

The work began with a study of the nuclear changes of *S. cæruleus* at time of fission, undertaken at the suggestion of Prof. E. L. Mark, when I was under his instruction at the Zoölogical Laboratory of Harvard University. The scope of the work was afterwards extended, and the investigation of several phases in the life-history of the Stentors was carried on at Williams College, at the Marine Biological Laboratory, Wood's Holl, Mass., and at Clark University, Worcester, Mass.

Material has been obtained from four widely-separated localities in Massachusetts : Cambridge, Williamstown, Falmouth, and Worcester. Each locality has yielded in abundance some

species or variety found very sparingly or not at all at the others, and only one form, *roeselii*, has been collected at all four stations.

Methods.

The study of Infusoria requires a special technique; and, as the wonderful results recently obtained by Maupas ('88, '89) forcibly show, an accurate acquaintance with the life-history and habits of the species under investigation is of the utmost importance. One of the most valuable aids in all biological and morphological research upon these organisms is the isolation of individuals for continuous study and experiment,—a method exceedingly simple in conception, but, owing to the minuteness of most Infusoria, often far from easy to carry out. This difficulty, however, hardly exists with the Stentors, which rank among the largest of their Class, and are easily visible to the naked eye.

In collecting, it has been my custom to bring to the laboratory as much as convenient of the natural surroundings of the Stentors—vegetable *débris*, sticks, stones, water-weeds, etc.—and a considerable quantity of the water in which the Stentors live. The gathering is put into shallow glass dishes, which are replenished with tap-water from time to time to make up for evaporation. Under these conditions *S. cæruleus* will usually thrive and multiply for a week or two; but owing to the rapid exhaustion of food, and to less well-ascertained causes, one cannot in this way maintain a permanent colony. *S. polymorphus*, *S. igneus* and *S. pyriformis* will usually live in aquaria much longer than the Blue Stentor; they, however, merely survive as individuals and seem never to increase in number.

In order to select individuals in fission or other conditions desired for study, I have used a simple contrivance by means of which large numbers can be rapidly reviewed. A slide is prepared by affixing a piece of sheet-wax a millimeter or less in thickness and about an inch square, in which circular holes have been punched. The holes are conveniently made with a cork-borer, and measure 3 mm. in diameter. The wax plate is fused to the slide by very cautious heating of both slide and

wax. A drop of water containing one or more Stentors is placed in each cell, and the specimens examined in turn with a low power of the compound microscope.

As to killing reagents, no reliable method has yet been found for fixing, in a state of extension, these highly-contractile Infusoria. Hydrochlorate of hydroxylamine, used in .25 per cent solution, neutralized with sodic carbonate, has been recommended by B. Hofer¹ as a paralyzing reagent, but I have tried it in vain on *S. cæruleus*. On the other hand, it seems impossible to find an agent of sufficiently rapid action to fix the animal before it contracts. Fortunately, contracted or semi-contracted specimens answer very well for most purposes. I have used for fixation, absolute alcohol, Flemming's chrom-aceto-osmic mixture, 1 per cent to .25 per cent osmic acid, Merkel's fluid, saturated aqueous solution of corrosive sublimate, and picro-acetic. The last two have proved the most satisfactory. The picro-acetic mixture I make up as follows: saturated aqueous sol. picric acid, 6 parts; glacial acetic acid, 1 part. It gives excellent results used either hot or cold, and should invariably be washed out with 50 per cent to 70 per cent alcohol.

In staining, two methods have been followed. First, for immediate study, one may fix and stain with a single reagent, such as an aqueous solution of methyl-green, to which a little acetic acid has been added; or Schneider's aceto-carmin. This method is invaluable for study of the structure of the meganucleus. Neither are lasting stains. For fixed and hardened material, intended for permanent preparations, I find that almost any of the carmine stains are good, while haematoxylin tinges the cytoplasm too deeply. I have used mainly Czokor's alum cochineal and picro-carmin, for they stain the cytoplasm least. In making preparations for study of the nucleus, decolorizing with acidulated alcohol is important, and should be carried far enough to extract all stain from the cytoplasm.

I have found it perfectly feasible to imbed Stentors in paraffine, and cut serial sections 5μ to 10μ in thickness. Sec-

¹ Zeits. f. wiss. Mikr., vii, pp. 318-326, 1890.

tions mounted in balsam, damar, or glycerine are essential for study of the micronucleus, particularly at times of fission and conjugation.

II. SYSTEMATIC AND FAUNISTIC.

The classification of the Stentors has always been difficult, on account of the great variability of most of the species. Consequently, different authorities have held very divergent views as to the number of species. Claparède and Lachmann ('59), for example, relegate the six forms described by Ehrenberg ('38) to two species, while Saville-Kent ('82) increases the number to nine. But the whole question of the specific distinctness of the different forms found in Europe has been so ably and fully discussed by Stein ('67) and his six species¹ have held their own so successfully that I see no occasion to alter his classification. Only one valid species, *Stentor auricula*, Sav. Kent, has been added to the European list since Stein; for I question very much whether *S. Barretti*, Barrett, will on further examination prove to be distinct from *S. roeselii*; and the various alleged "new species" of de Fromentel ('74) imperfectly described and too evidently based upon aberrant specimens, no longer hold a place in the system.

Our American Stentor fauna shows a surprising correspondence with that of Europe. With the exception of two marine forms, *S. multiformis* and *S. auricula*, every European species has been mentioned as occurring in this country.² In addition there seem to be two species (*S. globator*, Stokes ('85), and *S. pyriformis* sp. nov.) not found in Europe; but it must be admitted that neither of these species can be considered as fully established. *S. globator* is so aberrant that it might be regarded as based upon abnormal, dwarf specimens of one of the well-known Stentors, especially as the discoverer does not appear to have observed it more than once. While *S. pyriformis* is founded upon examination of a very large number of specimens, none of which showed transition to any known

¹ *S. polymorphus*, *cæruleus*, *roeselii*, *igneus*, *niger*, and *multiformis*.

² I have never myself seen *S. niger*; but it is given by Stokes ('88) in his list of American species.

species, it must be remembered that all the specimens came from a single locality; and I have found that Stentors, like many other animals of wide distribution, offer strongly-marked local varieties.

1. *Stentor igneus*, Ehrbg.

This species was found in abundance in two small ponds in Falmouth, Mass., during the summer of 1891. One of these ponds — a mere pool covering the bottom of a “kettle-hole” — is located near the village of Wood’s Holl. The pond is very shallow and choked with Sphagnum, among the fronds of which the Stentors live. The other pond was much larger, situated two miles from the first, near the village of Quisset. Here the Stentors were found among pond-lilies (*Nymphaea odorata* and *Brasenia peltata*) and other water-plants. They were not uniformly distributed, but colonized in certain places, apparently limited to the south side of the pond. This was probably owing to the fact that the other shores were wooded, and consequently shaded during a part of the day; for *S. igneus*, like all chlorophyll-bearing Stentors, is heliotropic.

This form agrees very accurately with Stein’s description and figures of *S. igneus*, and I have no hesitation in referring it to that species. The shape assumed by the animal in swimming varies from conical to pyriform. When attached and extended it assumes a trumpet-form very nearly like that of *S. polymorphus*, but not so attenuated. I have always found symbiotic green algæ (*Zoöchlorella*) present. The red pigment, however, is most frequently wanting. I have never found it in sufficient quantity to give a red tint to the animal as seen with the naked eye. The scantiness and frequent absence of pigment may be only a local or seasonal peculiarity, inasmuch as Leidy ('80) described a Stentor¹ from New Jersey in which the abundant pigment-granules imparted a crimson or lilac color to the animal.

As is well known, the meganucleus of *S. igneus* is a spherical or oval body, either single or multiple. Only small individuals,

¹ *S. amethystinus*, Leidy. I find nothing in his description, however, that would separate this form specifically from *S. igneus*.

so far as I have observed, have a single nucleus ; the majority have two, and many from three to five. Only once have I observed six. Stein ('67, p. 263) sometimes found three nuclei, but never a larger number.

1a. *Stentor igneus* var. *nigricans*, var. nov.

(Fig. 1; Pl. XXIII.)

In a little pond at Williamstown, Mass., I found countless numbers of a small *Stentor*, which seemed so different from all known species that for a long time I regarded it as new. The pond filled a slight depression in the open fields, covering about half-an-acre. It contained a scanty growth of aquatic grasses and rushes, but hardly any other vegetation. The *Stentors* were collected at various times during the fall of 1890 up to the time of freezing, and a few were kept in the laboratory through the winter. In the following April they were again obtained, at first in small numbers, but soon in the greatest abundance. By the middle of May they were so numerous as to give a dark coffee-color to the water of the pond, and completely cover all objects beneath its surface.

To the naked eye this form appears as a minute conical body, blackish or olive-green in color. Under the microscope the green tinge is seen to be due to symbiotic algae, while the blackish color is produced by a minute pigment in the ectoplasm. The meganucleus is conspicuous as a single, glistening body on the right side (Fig. 1, *mgn.*). When disturbed the animals swim rapidly with the usual rotatory motion for a long time ; but under quiet conditions attach themselves to fixed objects.

Later in the season I found at Wood's Holl the same *Stentor* in the small "kettle-hole" pond above mentioned, living in company with the typical *S. igneus*. The ordinary individuals of the two forms were so different that one could readily distinguish them by the naked eye. Not only do they differ in color, but also in shape and size. But after examining and closely comparing large numbers, I found individuals that could not be referred with certainty to either the type or its variety

nigricans. The new form must then be considered as specifically identical with *S. igneus*. But inasmuch as ordinary specimens of the variety differ so strikingly from the type, and even have in some cases a separate habitat, I consider it convenient to distinguish it by name.

Characters: *Small* (length, extended, .204 mm., diameter across frontal field, .136 mm.); *form, in extension, broadly conical or top-shaped, broadest across frontal field; posteriorly, abruptly tapering, curved, acute; when swimming and semi-contracted, form conical, pear-shaped or nearly cylindrical; when fully contracted, almost spherical. Membranellæ small and weak; striation of body and frontal field obscure; minute, brownish-black pigment in the ectoplasm; pellicula thin, easily ruptured. Contractile vacuole* (Fig. 1, c.v.) *on ventral side. Meganucleus single, spherical, 30 μ in diameter, usually lying near right side* (Fig. 1, mgn.). *Symbiotic algæ (Zööchlorella) always observed, usually abundant, indistinguishable from those of S. igneus and S. polymorphus.*

2. *Stentor pyriformis*, sp. nov.

(Figs. 2 and 3; Pl. XXIII.)

In October, 1891, I found a colony of green Stentors covering vegetable *débris* and water-weeds at the north end of Lake Quinsigamond, Worcester, Mass. At first I mistook them for unusually large specimens of *S. polymorphus*. But after extracting the chlorophyll with alcohol and staining, the meganucleus was found to be not moniliform, but in form of two or more entirely separate, spherical bodies (Fig. 3). This fact led me to examine the animals with care, and various points of difference between them and *S. polymorphus* were speedily discovered. The new form could not fairly be identified with any described species. It ranks as one of the largest of Stentors, being scarcely inferior to *S. cæruleus* in size. *S. pyriformis* never assumes the slender trumpet-shape of the higher Stentors, and in fact changes its form very little from the pear or conical shape it has while swimming (Fig. 2).

This species does not endure confinement so well as *poly-*

morphus, and I have been unable to keep them more than a month. Although I have examined a large number, I have never found one in fission.

In April, 1892, I visited another colony of *S. pyriformis* in Lake Quinsigamond, for information in regard to which I am indebted to my friend Dr. Wm. S. Miller. This colony is located in a winding, shallow bay known as "the Sanctuary." About two acres of the bottom are literally covered with these Stentors, which give it the appearance of being overgrown with a minute green vegetation. The Stentors live at various depths down to four or five feet. The earliest specimens collected (April 18) contained much less chlorophyll than those obtained in the fall, and in many the very narrow and obscure stripes were visible, which is never the case when the zoöchlorellæ are abundant. The specimens, furthermore, were inert, remained constantly contracted, and in many cases I could not distinguish the adoral zone. I believe, therefore, that they had recently emerged from the cyst. Stentors collected at the same place April 23 were more active, and contained a much greater quantity of zoöchlorellæ.

Characters : *Large (length, extended, .5 mm., breadth across frontal field, .2 mm.); in extension, funnel-shaped, elongate conical, or pyriform; in semi-contraction, pyriform or nearly cylindrical; anterior third of body of nearly uniform diameter, same as frontal field; posterior extremity obtuse, rounded; stripes very obscure, narrow (3.5μ in width) generally not visible in living animal; adoral zone narrow, membranellæ weak, oral aperture placed considerably posterior to aboral extremity of zone; endoplasm whitish, opaque, crowded outwardly with zoöchlorellæ; no pigment; contractile vesicle in usual position, to left of mouth (Fig. 2, c.v.); a single meganucleus rarely observed, nearly always 2-4, spherical or oval, 40μ in diameter. Hab., Lake Quinsigamond, Worcester, Mass.*

S. pyriformis obviously belongs to the group of small Stentors with simple, spherical meganuclei, and lowly-organized frontal field. Its nearest ally is evidently *S. igneus*, from which it differs in its much greater size, entire absence of pigment, thicker form when extended, and narrower stripes.

3. *Stentor roeselii*, Ehrbg.

(Fig. 4, Pl. XXIII.)

I find no essential difference between the American specimens of this form and the European *S. roeselii* so admirably figured by Stein ('67, Taf. VII, VIII). The meganucleus is often moniliform, it is true (Fig. 4, *mgn.*), but this condition has been found to obtain in the European form as well.

S. roeselii, like all its genus, is variable in size and shape. Specimens from Alewife Brook, Cambridge, are for the most part very small; while those found at Williamstown were unusually large, measuring when fully extended, 2 mm. in length and .19 mm. across the expanded frontal field.

This species frequents quiet waters where flocculent vegetable *débris* abounds, in the midst of which the so-called "sheath" is formed (Fig. 4, *sh.*). It is generally held that the sheath is due to a mucilaginous secretion. After observing carefully the habits of the animal, its surroundings, and the structure of the sheath, I am strongly inclined to the view that there is no secretion, but that the sheath is made up wholly of the slime and bacterial zoöglöea in the midst of which this *Stentor* delights to anchor itself. As I have observed it, the sheath is of the most indefinite form, and indistinguishable from the surrounding slime. As Stein long ago pointed out, the sheath has imbedded in its substance various minute foreign bodies (bacteria, diatoms, etc.), the presence of which is not favorable to the view that the sheath is secreted by the animal.

Although *S. roeselii* is perhaps the most sedentary of its genus, it is often seen swimming, when it assumes a clavate form, but slightly widened at the anterior end. On attaching itself, it expands into a graceful trumpet- or calla-like form (Fig. 4). In this condition *S. roeselii* is readily distinguishable from colorless specimens of *S. polymorphus* (the *S. Mülleri* of Ehrenberg) not only by its more slender form, but still more sharply by the characteristic shape of the frontal field. In fact, the frontal field of this species displays the highest development of any *Stentor*, and judged by this character alone, *S. roeselii* would rank the highest of the *Stentors*.

S. roeselii endures confinement better than the Blue Stentor, and I have kept specimens for months under seemingly very unfavorable conditions. They sometimes multiply to some extent in aquaria. The food consists largely of bacteria.

4. *Stentor polymorphus* (Müll.), Ehrbg.

The specimens of this species which I have studied do not differ in any important respect from the typical *S. polymorphus* of Europe. I found them in sparing numbers at Williamstown, and an immense colony at Worcester. This colony is established in a slow stream, from one-and-a-half to four feet in depth. The bottom is covered with large angular pebbles and small boulders. There is almost an entire absence of all except microscopic vegetation. For a distance of 100 yards the Stentors are attached to the stones in such numbers as to give to the whole bottom a mottled green appearance.

I have found not a single specimen of *S. polymorphus* entirely free of zoöchlorellæ, although some from Lake Quinsigamond contained very few, and appeared pure-white to the naked eye.

Although little inclined to multiply, this species bears confinement better than any other Stentor with which I am acquainted. One often finds them in very old gatherings that have stood in the laboratory for months.

5. *Stentor cæruleus*, Ehrbg.

This beautiful species is in all probability as widely diffused as the ubiquitous *S. polymorphus*, but seems to be more restricted to particular localities. With the exception of a very few found in Quisset Pond, Falmouth, and a few from Wolf Lake, South Chicago, Ill., my specimens have all come from Alewife Brook, Cambridge, where the species has multiplied exceedingly under rather peculiar conditions. Alewife Brook receives the sewage of a portion of North Cambridge, and also the escape-water from the engine of the pumping-station of the Cambridge Water-works. The water from the pumping-station warms the brook to such a degree that for a distance of 100 yards below its influx the brook freezes over only in the coldest

weather. This concurrence of favorable conditions causes the brook to swarm with all kinds of active aquatic life throughout the winter. I have obtained Blue Stentors at various points along the brook, and in every month from October to June. What becomes of them in summer I have never been able to determine. During July and August, 1890, I made numerous gatherings at many points along the stream, but without the least success.

The Blue Stentor of this country is undoubtedly identical with the *S. cæruleus* of Europe, but those I have obtained certainly much exceed the dimensions commonly given for the European form. Stein states ('67, p. 240) that the present species attains about the same size as *S. polymorphus*, the length of which in extension is given as half-a-line; *i. e.*, rather more than a millimeter. Maupas ('88, p. 230) gives as the "normal size," length 1.176 mm. and diameter .27 mm. In the semi-contracted condition of swimming the Alewife Brook specimens are commonly a millimeter long, while sessile and extended individuals generally attain a length of 2 mm. and a diameter of .476 mm. across the frontal field. Individuals in a state of extreme extension even reach a length of 4 mm. On the other hand, the largest measurement of *S. polymorphus* (fully extended) I have ever made was 1.466 mm. by .365 mm.

Stein remarks (p. 242) that the nodes of the meganucleus of *S. cæruleus* are usually spindle-shaped, while those of *S. polymorphus* are oval. I have frequently seen spindle-shaped nodes in the Blue Stentor, but oval nodes are much commoner.

III. MORPHOLOGY.

For the investigation of nearly all subjects relating to the morphology and physiology of the Stentors, *S. cæruleus* is by far the most favorable form. It is of large size; all its structural features are strongly accentuated; internal parts are readily seen in the living animal; it is obtainable in large numbers, and multiplies freely in aquaria. These advantages have been recognized by the different workers upon the Infusoria, and the result is that our knowledge of the Stentor type is very largely based upon the study of *S. cæruleus*.

A. ANATOMY.

The recent careful study of the external anatomy and the fission of the Blue Stentor by Schuberg ('90) added very much to our stock of information regarding this form. I have re-investigated every structure described by him, and our results coincide in nearly every particular. But, in regard to fission, our observations are at variance on one or two important points. I have been able, furthermore, to add a few details to our knowledge of this important process, especially in regard to the nuclear changes, which hitherto have received less attention than the cytoplasmic phenomena of fission.

1. *Ecto- and Endoplasm.*

Just as the fundamental Metazoan body has two cell-layers differing in function, so the unicellular body of a Protozoön is susceptible of division into two layers, ectoplasm and endoplasm. This division is by no means artificial, but is based upon a difference in structure and function. The differentiation of the two layers is not so conspicuous among the Infusoria as with the Rhizopods, mainly on account of the relatively extreme thinness of the ectoplasm, much the greater bulk of the cytosome being endoplasm. There is, besides, often an absence of delimitation between the ectoplasm and endoplasm. Such is the case with Stentor, where, however, the presence of ectoplasmic pigment, or of zoöchlorellæ limited strictly to the endoplasm, often helps to define the two. In *S. cæruleus* the ectoplasm contains abundant pigment, arranged in definite longitudinal bands, the "blue," or "granular" stripes ("Rippenstreifen" of Bütschli). Implanted in its substance are the contractile threads and the "roots" of the cilia. It is pierced by the basal plates of the membranellæ, which pass through it and some distance into the endoplasm. It is of denser structure and of a firmer consistence than the endoplasm.

An alveolar layer of the ectoplasm has been demonstrated by Bütschli, Schuberg, Schewiakoff and others in many Infusoria, both holotrichous and heterotrichous. If such a layer exists in Stentor, as seems probable, it is extremely thin, and its

structure so obscure as to escape detection. In *S. cæruleus* the structure of the ectoplasm is further masked by the abundant pigment, which it is impossible to remove. Notwithstanding these observational difficulties, Bütschli ('89, p. 1298, Fig. 14 *d*) has figured an alveolar structure for the ectoplasm of *S. cæruleus*; but after repeated efforts, I am still unable to make it out.

The functions of the animal that have reference to the outside world — sensation, locomotion, contraction — are performed by the ectoplasm and its derivatives, cilia and myonemes; those that concern the internal economy of the organism — digestion, assimilation, excretion, and secretion — are subserved by the endoplasm. An important exception is presented by the membranellæ, whose two functions, capture of food and locomotion, certainly have reference to the outside world; and yet, contrary to the general opinion, I have found them to be derivatives of the endoplasm.

2. Ectoplasmic Structures.

Pellicula.¹—In common with the Ciliata generally, the Stentors possess an external limiting membrane which corresponds morphologically to the cell-membrane of pluricellular animals. While the pellicula confines the soft cytoplasm and helps to give the cytosome a definite form, it is at the same time wonderfully elastic and flexible, and admits of the striking mobility characteristic of all species of the genus. In the living animal the pellicula is not very apparent as a distinct structure, although brought into evidence by certain folds or wrinkles which appear most frequently at the anterior end, and by numerous minute transverse crenulations along the blue stripes, often seen when the animal is contracted (Fig. 28).

After treatment with .25 per cent. osmic acid the pellicula becomes raised from the ectoplasm along the granular stripes, but remains adherent at the clear stripes (Fig. 5, *pl.*). It is then seen to be a thin, structureless membrane.

¹ This term was first used by Bütschli to replace the older and inappropriate name *cuticula*.

Stripes.—In all species of Stentor the body and frontal field are adorned with alternate bright ("Zwischenstreifen" of Bütschli) and granular ("Rippenstreifen," Bütschli) stripes. Those of the body have a longitudinal course from the adoral zone to the foot; while the frontal stripes sweep in a left-handed spiral from the aboral portions of the zone, over the whole frontal field, and down the pharyngeal funnel to the oral aperture (Figs. 33, 37, 40). As we shall see later, the frontal stripes are the modified derivatives of stripes on the ventral surface.

The stripes are due to a difference in structure in the ectoplasm, the protoplasm of the bright stripes being more hyaline than that of the granular bands. The striation is naturally most conspicuous in species that possess pigment, for this is restricted to the granular stripes. Where pigment is absent and the endoplasm is rendered opaque by symbiotic algae, as in *S. polymorphus* and *S. pyriformis*, the stripes are generally not seen at all in the living animal, but may be demonstrated by treatment with aceto-methyl-green, aceto-gentian-violet, or osmic acid. If specimens of the same species, however, happen to be nearly free of chlorophyll, the stripes are visible in the living animal. In *S. roeselii*, again, the stripes are obscure, owing to the slight differentiation between hyaline and granular portions.

The only species I have found suitable for the study of the stripes is *S. cæruleus*. Here their course can be followed with comparative ease. The relative width of granular and bright stripes varies exceedingly in different parts of the body, and the frontal granular stripes are always much narrower than the granular stripes of the body. The relative width of granular and clear stripes at a point just under the adoral zone is seen in Fig. 6, *g. s.*, *c. s.* Here the granular bands measure 22μ , the clear bands about 7μ in width; but the average for the whole body would be considerably less. It is worthy of note that the gradual diminution in the width of stripes from the anterior to the posterior end of the animal takes place almost wholly in the granular stripes.

It was long ago pointed out by Stein ('67, p. 227), and has since been noted by Brauer ('85), Gruber ('86), and Schuberg

('90) that the course of the stripes in *S. cæruleus* is not always perfectly uniform from the anterior to the posterior extremity, but that forkings or breaks occur here and there. Brauer even mentions a particular clear stripe "auf der einen Seite gelegen, welche viele, bis 10, übereinanderstehende Seitenzweige abgab." But it remained for Schuberg ('90) to discover that a V-shaped area of branchings is present as a normal structure on the ventral aspect of *S. cæruleus*, and also to point out the interesting relation it bears to the anlage of the new adoral zone in fission. He thus describes this "ramifying zone" (p. 200): "Innerhalb zweier Streifen, welche continuirlich vom Hinterende nach vorne ziehen, findet man andere, welche zwar am vorderen Ende beginnen, das hintere jedoch nicht erreichen. Die ganze Zone, innerhalb welcher dies geschieht, sei 'Verästelungszone' genannt, der Streifen aber, welcher sie rechts begrenzt, sei als 'rechter,' der links als 'linker Grenzstreifen' bezeichnet. Beide Streifen beginnen am Hinterende nebeneinander; der rechte zieht von hier in ziemlich geradem Verlaufe bis zu der Stelle, wo die adorale Zone . . . dorsalwärts umbiegt, um in die Tiefe zu steigen, während der linke ungefähr in der Mitte der linken Körperseite auf die adorale Zone stösst, also etwa an der Stelle, wo After und contractile Vacuole liegen. . . . Die Streifen nun, welche innerhalb dieses Dreiecks verlaufen, entspringen sämtlich mit ihrem Hinterende mittelbar oder unmittelbar als rechtsseitige Abzweigungen des 'linken Grenzstreifens.'"

I have found the ramifying zone very constantly present in the Blue Stentor. In a few cases, however, careful examination has failed to reveal it; and in other species the striation is so obscure as almost to preclude its discovery. Branching of stripes is by no means confined to the ramifying zone, but its appearance on other parts of the body is adventitious and different in origin. As Gruber ('86) has remarked, these places are often the scars of former wounds, and can be artificially produced. It is doubtful, however, whether single forkings like those represented in Fig. 33 are so produced. I think it probable that these are formed in the same way that stripes

are multiplied in the new adoral zone at time of fission, *i.e.*, by the intercalation of a new clear stripe.

Myonemes.—Standing in close relation to the stripes are the contractile elements, called by Bütschli *myonemes*. One of these underlies each clear stripe, and thus subtends a row of cilia (Figs. 5, 7, 9, 12). In the living Stentor the myonemes are hyaline, flexible, and exceedingly contractile fibrils; in a Stentor killed with osmic acid or corrosive sublimate, they become rigid, highly-refractive rods (Fig. 11). Bütschli ('89, p. 1298) describes the myonemes of *S. cæruleus* as being oval in transverse optical section; to me, however, the section has always appeared to have a circular outline (Fig. 5). Neither have I been able to make out the transverse striations figured by Bütschli, even with the 2 mm. apochromatic homogeneous immersion of Zeiss. While I do not assert the absence of the striæ, owing to my inability to see them, I would nevertheless suggest that the appearance observed by Bütschli was of artificial origin.

Myonemes attain their largest size in *S. cæruleus*, where they are plainly visible in the living animal, if it be sufficiently compressed and examined with a power of 500 diameters. In other highly contractile Stentors, as *S. polymorphus* and *S. roeselii*, the myonemes are very distinct after application of reagents. In *S. pyriformis*, on the contrary, where the contractile power is much less, the myonemes are exceeding minute (Fig. 9, *mn.*).

The myonemes are to be regarded as highly-specialized differentiations of the ectoplasm, endowed with a contractile power not less than that of striped muscle. In both cases, the highly developed contractility undoubtedly has its origin in the comparatively feeble contractile power of protoplasm. Engelmann's ('75) beautiful researches on the contractile substances of the Protozoa have shown that the general ectoplasm of Stentor is anisotropic, and therefore probably contractile. He assigns to it the slow and gradual contraction one often sees in Stentor, so different from the lightning-like shortening effected by the myonemes.

It is exceedingly interesting to watch the action of the

myonemes in a strongly-compressed Stentor as they alternately extend and contract ; and no one who has once observed them under these conditions can doubt that they are responsible for the contractions of the animal. Lieberkühn ('57) was the first to observe the contraction of the myonemes, and it has since been very accurately and minutely studied by Engelmann ('75). Lieberkühn thus describes their action :

“Es sind scharf contourirte körnchenfreie Fasern etwa von der Breite der körnchenfreien Zwischenräume, unterhalb deren sie der Längsaxe des Körpers nach verlaufen ; sie setzen sich vorn unter dem grossen Wimperkreis und hinten am ‘Saugnapf’ an ; einige von ihnen vereinigen sich während ihres Verlaufs. Am deutlichsten sieht man die bei der Contraction eintretenden Veränderungen, wenn ein farbloser oder wenig farbiger Stentor gerade so liegt, dass man auf den kreisförmigen Saugnapf blickt ; man sieht alsdann von seinem Umfang im Zustand der Ruhe alle einzelnen Muskeln geschlängelt abgehen ; in demselben Moment aber, wo sich das Thier zusammenschnellt, also verkürzt, verschwindet die geschlängelte Form vollständig, die Muskeln strecken sich gerade.”

As Lieberkühn states, the myonemes extend from the adoral zone to the “sucking-disc,” or foot. When in the contracted state they are much thicker posteriorly than anteriorly (Fig. 11), probably because the most contraction takes place in the former portion. They always remain very indistinct towards the adoral zone, and although I have been able to trace them up to it, I have not seen the manner of their ending there. At the other extremity, however, the termination of the myonemes is evident enough (Fig. 12). They end abruptly just at the edge of the pellicula bordering the naked protoplasm of the foot.

In the ramifying zone not only the clear stripes branch, but also the myonemes underlying them, as I have sketched in Fig. 10. The myonemes are represented in the extended condition, and are therefore thrown into folds. The presence of these convolutions in the myonemes, entirely independent of any corresponding folds in the ectoplasm, indicate that there is no firm connection between the two. The convolutions are

so pronounced in the posterior part of the animal at the moment of extension that they completely underlie the granular stripes, as Stein has beautifully shown in one of his figures of *S. roeselii*. This would be impossible if the myonemes were bound firmly in place beneath the clear stripes. Bütschli ('89, p. 1298) has represented the myoneme of *S. cæruleus* as lodged in a "canal," lying in the "alveolarschicht" beneath the clear stripe. Although I have searched for it repeatedly, I have been unable to find the least evidence of such a structure, either in optical or actual sections.

Pigment.—Three distinct pigmentary substances are present in the Stentors: a blue pigment in *S. cæruleus* and *S. multiformis*, a brown pigment in *S. niger* and *S. igneus nigricans*, and a purple-red pigment in *S. igneus*. The pigment is, for the most part, lodged in the ectoplasm, and restricted (at least wherever its position can be accurately determined) to the granular stripes. It is thus placed in a position to receive the greatest amount of light.

The pigment of *S. cæruleus* is certainly one of the most remarkable of animal pigments. Under normal conditions it varies in tone from bright sky-blue to pale sea-green, and even to a dull bluish-gray. When individuals are kept in unfavorable environment, as under a cover-glass, the pigment becomes reduced in quantity, and changes to a yellowish-brown color; sometimes Stentors are almost wholly devoid of it, and appear nearly pure white. Schuberg ('90, p. 221) noted that the loss of pigment took place by excretion, and I have frequently observed the same fact. In a culture where Stentors are undergoing depigmentation, numerous bluish-gray clots are invariably found, composed largely of pigment granules. Schuberg often observed pigment in the excrement vacuole, and appears to be of the opinion that it is excreted in the same manner as the faecal matter. I have frequently seen such pigment-containing vacuoles, but do not feel sure but that the pigment they hold often belongs to a small Stentor that has been swallowed as food. Furthermore, many of the clots of pigment in a culture may be due to the death and disintegration of the feebler members of the colony. But by starting a

small colony and keeping a rigid census of it, it is easy to ascertain that pigment is thrown out of the bodies of living Stentors. As Schuberg remarks, pigment accumulates about the foot of Stentors that remain long fixed in one place; it is evidently thrown out by the naked protoplasm of the foot.

The blue coloring-matter of *S. cæruleus* is perhaps the most indestructible of all animal pigments. Bütschli observed ('89, p. 1476) that neither alcohol, ether, nor chloroform dissolve it, and Lankester ('73) found it unchanged by dilute acetic, hydrochloric, and sulphuric acids, while an alkali (dilute potassic hydrate), "had the effect of intensifying the blue color." The only reagents I have used that attack it are osmic acid and platinic chloride, both of which change its color to brown.¹

Cilia. — The whole surface of the Stentors, in common with all other Heterotricha,² is clothed with cilia. As previously stated, the cilia are inserted in rows along the clear stripes (Figs. 5, 7, *cl.*). The narrower the granular stripes, the nearer together the rows of cilia; consequently the ciliation is densest in the pharyngeal funnel, where the stripes are the narrowest of any on the body.³ The cilia are not of equal length on all portions of the body. Those of the frontal field are shorter than those of other parts; and they are somewhat longer on the posterior than on the anterior regions of the body.

The structure of the cilia does not appear to differ from that of these organs among Metazoa, although I have not been able to detect a "root" within the pellicula. A thickened basal piece ("Fussstück") is, however, readily demonstrable by use of osmic acid. (Fig. 7). The absence of a "root" is not to be assumed because of failure to detect it; such a structure has been shown to exist in nearly all cilia and ciliary structures.

¹ The only recorded effort to determine the chemical composition of this remarkable substance is the spectroscopic examination made by Lankester ('73). Its spectrum indicated the presence of a peculiar substance, called by Lankester *Stentorin*. It is characterized by the presence of two strong absorption bands, one in the red, the other in the green. "Stentorin is interesting as being an addition to the very short list of animal substances which give banded and therefore characterizable absorption spectra."

² The aberrant *Cænomorpha* alone excepted.

³ This fact is readily demonstrated by compressing a Stentor beneath a cover-glass sufficiently to cause eversion of the pharynx.

Its invisibility here is possibly due to the proximity of the underlying myoneme.

The occurrence "tactile spines" ("Tastborsten") in *S. caeruleus* and *S. roeselii* has been mentioned by Stein and others. To account for the suddenness of their appearance in places where none could be seen before, Stein expressed the view that they could be withdrawn into the body and again suddenly protruded. I have frequently observed these so-called "spines" in *S. caeruleus* and occasionally in *S. roeselii* (Fig. 4, right-hand outline). They are more frequently seen about the anterior end of the animal, when one is looking directly down upon the frontal field. I have often noted their sudden appearance and disappearance. It is my strong belief that these "spines" are simply cilia that have become momentarily rigid, and therefore visible, while their disappearance is due to the fact that they have resumed their rapid swinging motion, in which condition they are not individually discernable. I have not seen any "spines" so large as many figured by Stein, and their length was certainly not greater than that of cilia. In drawing such structures, however, it is an easy matter to exaggerate unconsciously their size, and this is probably the case with Stein's figures.

While it is perhaps idle to speculate on the function of temporarily rigid cilia, the view that they are improvised tactile organs seems the most reasonable. The anterior border of the animal, which is brought most frequently into contact with foreign objects, is naturally the most in need of tactile organs, and it is just here that I have most frequently observed the "spines." It is well-known that permanently rigid spines or bristles occur in many Infusoria, notably the *Hypotricha* (*e. g.*, the three caudal spines of *Stylonichia mytilus*), and there is every reason to believe that these possess a tactile function. All such spines are undoubtedly modified cilia. It would seem, then, that we have in the temporarily rigid cilium of *Stentor* an organ which is virtually a cilium, but having acquired enhanced tactile sense (probably possessed in some degree by all cilia), and the power of becoming rigid, is already on its way to become a specialized organ of touch.

3. Pharyngeal Funnel.

That portion of the frontal field that sinks spirally into the animal was regarded by Stein and later writers as the oesophagus, and its external aperture as the mouth. But Schuberg ('90, p. 202) has shown conclusively that the whole funnel is an in-turned portion of the frontal field, and that the real mouth lies at its innermost extremity (Figs. 35, 62, *o*). Schuberg has very accurately figured (Taf. XIV, Figs. 2, 3) the appearance of the pharyngeal funnel of *S. cæruleus* in the contracted state. With the extension of the animal, the funnel undergoes great enlargement, and the outer turn, or buccal pouch (Figs. I, 33, 62, *b.p.*) ("Peristomtasche" of Stein)

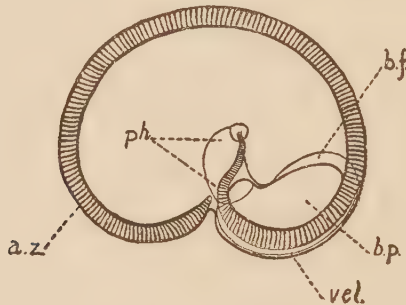


Fig. I.—Diagram of frontal field and pharynx of *S. cæruleus*, expanded. *a.z.*, adoral zone; *b.f.*, buccal fold; *b.p.*, buccal pouch; *ph.*, pharynx; *vel.*, velum.

sometimes attains nearly one-half the area of the whole frontal field. It serves as a very capacious "hopper" for whatever grist is brought by the "alimentary vortex." If one looks directly down upon the frontal field, the buccal pouch is seen to have an outline varying from almost circular in state of utmost expansion, to reniform and crescentic according to the amount of distension (Fig. I, *b.p.*). It also varies much in different individuals. The concave side of the crescent is always dorsal; the convex outline is formed by the "hypostom" of Stein. This hypostom is the thin raised edge of the ventral body-wall, crowned with the adoral zone as it passes towards the right to enter the funnel. Inasmuch as the name

"hypostom" is based upon the wrong assumption that the mouth is at the entrance of the funnel, it is desirable to give this structure a new designation, and I propose the term "velum" (Figs. I, 33, *vel.*). The velum is covered outwardly by the pellicula of the anterior portion of the body and internally by that lining the buccal pouch. It is probable that the ectoplasmic layers of the two surfaces are in contact, but when contraction takes place the endoplasm flows in between the surfaces, and the velum is almost or even wholly obliterated.

The descent from the higher and more aboral portion of the frontal field into the buccal pouch is very sharp and sudden. At the left the pouch curves dorsalwards and forms a pocket which is partially covered by a reduplication of the frontal field—the "buccal fold" (Fig. I, *b.f.*),—and by the adoral zone. This is the beginning of the "Rinne" of Schuberg ('90, p. 203), which he figures as a channel starting from this point and traversing the ventral side of the pouch towards the mouth. This channel, however, is almost entirely obliterated in the fully-expanded condition of the buccal pouch. At its right extremity the pouch narrows suddenly, and passes inward and dorsalward to form the inner turn of the pharyngeal spiral, or pharynx proper (Fig. I, *ph.*).

The foregoing description of buccal parts applies particularly to the Blue Stentor. These parts are, however, almost equally well-developed in *S. roeselii* and *S. polymorphus*; in both species the buccal pouch is especially prominent. In the lowly-organized forms, such as *S. igneus* and *S. pyriformis*, no buccal pouch is present. (Figs. I, 2.) Allied genera, as *Climacostomum* and *Folliculina*, also appear to be destitute of a buccal pouch, so that this structure may fairly be considered as developed among the Stentors, and only in the higher species of the group.

4. Endoplasmic Structures.

Membranellæ.—It was first pointed out by Sterki ('78) that the adoral zone of the Heterotricha (Stentor) and Hypotricha is composed, not of large cilia as held by Stein ('67) and Simroth

('76), but of flat, transverse plates, called by Sterki "membranellæ." His observations have been confirmed by all subsequent writers upon these structures. Schuberg's careful description of the membranellæ of *S. cæruleus* relieves me from giving a detailed account of them. I have studied them as independently as I could, and my results agree with his at almost every point.

When the membranellæ are in rapid motion the impression is that of large, strong cilia, each inserted at the lower extremity of a glistening rod extending across the adoral zone. The same appearance is very strong when the membranellæ are moving slowly, and after a few observations of this sort it is almost impossible to avoid forming the conclusion that one has to do with large cilia. But in order to obtain a true conception of them, their motion must be arrested and a side-view obtained. By bringing sufficient cover-glass pressure upon the adoral zone, the membranellæ are flattened down and made to overlap one another, all motion being reduced to a slight tremor (Fig. 6, *m.*). Then the whole outline is seen.

After fixing with corrosive sublimate or other suitable reagent, the membranellæ may be studied either in optical section or actual ones, and both extra- and intrapellicular portions of the membranellæ may then be observed. (Figs. 8, *m.* and 13, *a.s.*¹) In side view, as in Fig. 8 (where the section cuts the newly-formed adoral zone transversely) the basal plate (*b.p.m.*) and its terminal filament (*t.f.*) are plainly seen. When the zone is examined in longitudinal optical section (Fig. 13), a third intrapellicular structure is visible, the connecting filament (*c.f.*),—the "Basalfibrille" of Schuberg. By focusing through the adoral zone, this fibre is also seen in the living animal. Another structure plainly seen, is the thickened and highly-refractive basal portion ("Basalsaum" of Schuberg?), Figs. 13, 30, just outside the pellicula. This I regard as homologous with the "basal piece" (*Fussstück*) of a cilium, and has in young membranellæ precisely the same appearance as in cilia. Although I have not traced it throughout the development of the membranellæ, there can be little

doubt that it becomes the highly-refractive "basal seam" that forms so conspicuous a portion of the membranella.¹

The membranella is generally regarded as having originated by the fusion of cilia, and the ease with which it splits up longitudinally on application of reagents (see Fig. 8, *m.*) is held to be evidence of this. Besides, it is possible to homologize every part of the membranella, excepting the connecting filament, with parts already present in cilia. Furthermore, there are forms among the Holotricha (*e.g.* *Didinium balbianii* and *Dinophrya lieberkuehni*) in which Schewiakoff ('89) has figured ciliary organs which might fairly be considered as incipient membranellæ. He thus describes them in *Didinium balbianii* (p. 15): "Am Rande des abgestutzten Vorderendes befindet sich ein Kranz ziemlich langer Cilien, welche in kleinen Reihen sehr dicht angeordnet sind. Dieselben erscheinen auf den ersten Blick membranellenartig und an der Spitze zerfasert; es fällt aber nicht schwer sich zu überzeugen, dass es einzelne Cilien sind, gewöhnlich 6 an der Zahl, welche sehr nahe aneinander stehen und an der Basis wie verklebt erscheinen."

The function of the connecting filament, which binds together all the membranellæ of the adoral zone, is still very obscure. Brauer ('85), who appears to have been the first to mention the connecting filament, ascribed to it a contractile function, believing that it served to contract the frontal field; but Schuberg has very properly pointed out that this function is amply provided for by the spiral myonemes of the frontal field. On the other hand, the view that this intraplasmatic apparatus has a nervous function (first suggested by Engelmann, '80, for the inward prolongation of the cirri of *Stylo-nichia*) seems probable, but incapable of proof. The connecting filament in Stentor, as far as position goes, is admirably adapted to coördinate the action of the membranellæ, whose

¹ The "bilamellose" structure of membranellæ was first made out by Schuberg ('86) in the membranellæ of *Bursaria truncatella*, and was afterwards reported by Bütschli and Schewiakoff ('89, p. 1335), and by Schuberg himself ('90), to be present in those of Stentor. In transverse optical section the appearance is that of two parallel rows of dots—the optical expression of two fibrillated lamellæ. I have not been able to make out this structure in the membranellæ of *S. cæruleus*.

uniform vibration is essential to the production of an alimentary vortex.

The Foot, or organ of attachment, is beautifully adapted for anchoring the animal while it expands its frontal field. Attachment is accomplished, not by means of a "sucker" as believed by Stein and all the earlier writers, but by pseudopodia protruded from the naked disc of endoplasm (Figs. 14, 15, 16). The extent of protrusion and consequently the whole aspect of the foot depends upon the nature of the substance to which the animal is affixed. Thus Fig. 16 shows the foot of *S. polymorphus* attached to glass; pseudopodia are not protruded, but the whole foot is simply flattened against the glass, and sticks to it apparently by virtue of the intrinsic adhesiveness of the protoplasm. Fig. 15 is a sketch of the foot of *S. roeselii* fixed in loose and flacculent detritus. Spine-like pseudopodia are protruded in all directions; they are mostly unbranched. Fig. 14 represents the very beautiful, ramose pseudopodia of a Blue Stentor affixed to the surface film of water (in this case covered with a very thin bacterial zoöglöea). Although the figures are taken from three species, the aspect of the foot represented by each, together with an almost infinite series of intermediate forms, may be found in any one species.

A Stentor never becomes attached until the "stalk" is well protruded from the body. If the foot be carefully watched when the animal is preparing to affix itself, numerous minute processes in rapid vibration will be seen. These bear the closest resemblance to cilia; indeed, I have often been unable to distinguish, so far as the form of the structures is concerned, where the ciliary covering of the body ends, and the pseudocilia of the foot begin. They offer an interesting case of a ciliary organ on naked protoplasm. These minute processes are in all probability highly sensitive, and enable the animal to feel for a suitable place of attachment. Stentors appear to experience difficulty in fastening on clean glass. I have often watched one fully extended, dragging its foot over the under surface of a cover-glass before being able to fasten upon it. If a number of Stentors are put in a glass dish of water, very

few affix themselves to the sides of the dish until the glass has become coated with slime.

The Nuclei.

Meganucleus.—The three principal forms in which the meganucleus of the Ciliata occurs—the spherical or oval, the vermiform, and the moniliform—are present among the Stentors. All the lower forms (*S. igneus*, *S. multiformis*, *S. niger* and *S. pyriformis*) have simple, rounded meganuclei. The higher forms (*S. auricula*, *S. cæruleus*, *S. polymorphus*, and sometimes *S. roeselii*) have moniliform nuclei. *S. roeselii* retains the vermiform nucleus for a long time after fission, but the moniliform condition is eventually attained.

The spherical meganucleus is evidently the primitive form, and the vermiform and moniliform shapes are derived from it. The stages of what must have been its phylogenetic development are repeated at every fission. The vermiform nucleus is not permanent among the Stentors. Even in *S. roeselii*, where it is of such frequent occurrence as to have been taken for a diagnostic feature by Ehrenberg ('38) and even by Stein ('67), it appears to be only a transient condition, readily passing into the moniliform state if a considerable time elapses between periods of fission (Fig. 4, *mgn.*).

The moniliform nuclei differ but slightly from one species to another. The meganucleus of *S. roeselii* is peculiar in that the anterior nodes are the thickest and largest, while the smaller posterior ones are long and spindle-shaped (Fig. 4). This results from the shape of the vermiform nucleus, which is always thickest anteriorly. Although the size and shape of the nodes vary considerably in *S. cæruleus*, and also the length and thickness of the commissures, I have but rarely noted any marked deviation from the typical form. One of these was an imperfectly-noded nucleus of a small individual (Fig. 17), which had but three well-defined constrictions, producing four elongated, unequal nodes, two of which showed indistinct constrictions indicating where commissures would normally be. The condition is evidently one of arrested development, for such imperfectly-noded nuclei are by no means rare at the

later stages of fission. The other instance of abnormal meganucleus is shown in Fig. 18. We here have the rare anomaly of a nucleus with a side-branch. The animal was killed at a late stage of fission, just as the nucleus was returning to the moniliform condition. If the process of nodulation had been completed, we should have had a noded branch such as Stein has figured ('67, Taf. V, Fig. 8) for *S. polymorphus*.

The number of nodes present in the meganucleus of one and the same species is well-known to be highly variable, but within definite limits. Thus in *S. polymorphus* I found only two meganuclei out of fifty which had fewer than 8 nodes (these had 6 and 7 respectively); and only two that had as many as 18. The average of the fifty examples was 12.42 nodes.¹ This agrees with Stein's ('67, p. 231) statement that the nucleus of *S. polymorphus* most frequently has 11-13 nodes, and that he only once found a nucleus that had as many as 20 nodes. The meganucleus of *S. cæruleus*, according to my experience, regularly has more nodes than that of *S. polymorphus*; but Stein (p. 242) found, on the contrary, a smaller number, and never counted in any meganucleus more than 13 nodes. Later observers, however, have frequently found 20 or more nodes in European specimens of *S. cæruleus*. I have counted the nodes in 75 stained specimens of this species, and have found the maximum number to be 20, which occurred but twice.² The minimum number was 9, found only once; but occasionally a smaller number is found in this species (see p. 524). The average number of nodes for the 75 examples was 14.16.

The finer structure of the infusorian meganucleus has been frequently described. It is well known that its appearance is strikingly different from that of the typical nucleus, and it has been appropriately termed "massive" in contradistinction to the "vesicular" nuclei of Metazoa and of plants. The nuclear substance of Stentor is dense, granular and viscous. It is enclosed by a firm membrane, very conspicuous in isolated

¹ The specimens in which the nodes were counted were taken at random and came from both Williamstown and Worcester.

² I have, however, seen as many as 22 nodes in this species (see p. 524).

nuclei killed and stained with aceto-methyl-green and examined in water or glycerine. The presence of a nuclear membrane in the meganucleus of Infusoria was denied by Jickeli ('84), but evidently on insufficient grounds. In the living condition the membrane fits closely over the nuclear substance.

In the meganucleus of all species of Stentor studied by me, I have observed, in addition to the granular chromatin, a minute, rather obscure chromatic network, which takes with all nuclear dyes a decidedly deeper stain than the granular chromatin (Figs. 8, 19-21, 60). This is evidently the structure seen and figured by Carnoy ('84) in *S. polymorphus*, and believed by him to be a much-involved chromatic filament. It has appeared to me, on the other hand, that the meshes are "closed," and that we have here to do with a chromatic "network." The observational difficulties of solving the problem are so great that it seems impossible to overcome them with our present means of investigation.

The chromatic network appears to be of wide occurrence in the meganuclei of the Ciliata. It has been figured by Schewiakoff ('89) for *Holophrya discolor*, *Didinium balbianii*, and *Dinophrya lieberkuehnii*. Carnoy found it in Stentor and in Vorticella. I have seen it very distinctly in *Spirostomum teres*, and less so in *Paramecium caudatum*. It is sometimes visible even in the living nucleus of *S. cæruleus*, so that it cannot be regarded as the effect of the reagent or of post-mortem change.

The chromatic network always presents the same aspect of delicate meshes composed of minute, wavy, granular-looking threads. It is not peripheral, but, as focusing and sections (Fig. 8, *mgn.*) show, runs all through the nucleus, and has the same appearance from whatever direction it is seen. It is evident, then, that the "network" is a web having three dimensions.

The network undergoes no visible change at time of division. Constricted nuclei (Fig. 21) and moniliform nuclei in a state of condensation (Fig. 8, *mgn.*) do not reveal any modification of it. I have never seen the least indication of a "striation" or linear arrangement of threads in any constricted nucleus of Stentor, such as Balbiani ('60), Bütschli ('76), Carnoy ('85),

Gruber ('84^a), Jickeli ('84), and others have described as occurring in various Infusoria. In preparations of *Paramecium caudatum*, showing that form at many stages of fission, I have been equally unsuccessful in finding a longitudinal arrangement of chromatic threads. Furthermore, in R. Hertwig's ('89) beautiful memoir on the conjugation of *Paramecium aurelia*, a figure of that species in fission is given, but the strongly constricted meganucleus has no trace of striation. I believe, therefore, that any attempt to regard the longitudinal arrangement of chromatic threads a constant feature of meganuclear division, and adduce it as evidence of a disguised mitosis, is unwarranted.¹

In moniliform nuclei both chromatic granules and network are restricted to the nodes, and do not extend into the commissures unless the latter are unusually thick (Figs. 19, 44, 45). In any case, the commissures doubtless contain a thread of the non-stainable, semi-fluid portion of the nucleus, the karyoplasm. The karyoplasm is so charged with granular chromatin that it is seldom distinguishable as a separate substance. The translucent oval bodies, looking like vacuoles, which are frequently present in the meganuclei of all Stentors (Figs. 19, 20), are probably to be regarded as segregations of karyoplasm free from chromatin. Each globule occasionally contains a minute, slightly-stainable, denser, and refractive body, the nature of which I am ignorant (Fig. 20).² It is not a micronucleus, for it frequently lies deep within the meganucleus.

An interesting fact in regard to the meganuclei of *S. igneus* and *S. pyriformis* is that they undergo division independently of that of the cytosome (Figs. 22-24). Indeed, the vast majority of individuals of both the above-named species have two or more entirely separate meganuclei. I have, besides, found nuclei in every stage of constriction. Owing to the frequent occurrence among the Ciliata of moniliform nuclei with very attenuate commissures, it has been held by some

¹ In a previous article ('92, p. 155) I have taken the ground that the appearance of longitudinal threads in a dividing nucleus is not *in itself* evidence of mitosis.

² This is evidently the structure figured by Stein in the nodes of *S. polymorphus* ('67, Taf. V, Figs. 7, 9, 12).

writers that, except in a very few instances (*e.g.*, *Opalina*), multiplicity of meganuclei does not obtain in this group. Even where the meganuclear substance has every appearance of being broken up into an immense number of separate elements, as in *Urostyla* (Bergh, '89), *Holosticha* (Gruber, '88), *Holophrya* (Maupas, '83), and other forms, it is maintained by Balbiani, Bütschli, and others that the apparently separate elements are all nodes of an immensely long and much-convoluted meganucleus, the commissures of which are so exceedingly fine as to escape observation. I am, however, of the opinion that complete meganuclear division does occur among the Ciliata independently of fission. Among the Stentors, commissures are comparatively thick and conspicuous, so that there is never the least difficulty in making them out. Furthermore, the node is tapered more or less where it is produced into the commissure (Fig. 19). But in *S. pyriformis* and *S. igneus* there is neither any trace of commissures nor of a tapering of the meganuclei (Figs. 3, 23). As long as the daughter-nuclei remain in connection, the constricted part is perfectly distinct, (Fig. 24) and absolutely no evidence has been found of its being drawn out into an exceedingly slender commissure.

Perhaps the strongest evidence that can be adduced in favor of the presence of invisible commissures joining the meganuclear elements, is the consolidation of all the nodes into a single mass at time of fission. It appears to me extremely doubtful whether this takes place when the nuclear elements are wholly separate. As all observations on the condensation of unquestionably moniliform nuclei show, the concentration takes place by a widening out of the commissures; and from watching the changes in the living meganucleus of *S. cæruleus*, I have been impressed by the fact that the nuclear membrane effectually prevents coalescence of separate nuclei, even when they are strongly pressed together (see p. 514). It would, therefore, be very interesting to observe the behavior of the meganuclei in *S. igneus* or *S. pyriformis* at time of fission. This unfortunately I have not been able to do, owing to the great rarity of fission in these forms when kept in confinement.

What purpose is subserved by the multiplication of meganuclei in the infusorian body? It seems to me that it is best referred to the same cause as that productive of branched, moniliform, and vermiform nuclei, both among Protozoa and Metazoa. I have elsewhere ('92, p. 138) expressed the view, in agreement with Chun, that one of the prime motives for amitotic division is the distribution of nuclear material through the cytoplasm, and the occasion is especially urgent where the mass of the nucleus (as is the case with *Stentor*) is small in comparison with the mass of the cytoplasm. A point of interest in this connection is the very frequent contemporaneity of meganuclear division when there are two or more meganuclei (Fig. 24). It is the usual thing to find both nuclei at nearly the same stage of division, but not infrequently one will be found to have outstripped the other, so that specimens occur with two separate nuclei and a third constricted one. This indicates a response on the part of both nuclei to the needs of the cytoplasm.¹

Micronuclei.—The micronuclei of the *Stentors* have been among the most difficult to demonstrate of all the Ciliata. They escaped the scrutiny of all the earlier micrographers, and were first seen by Balbiani ('61) at time of conjugation, when they become greatly enlarged. But at that period Balbiani was no more successful than his predecessors in discovering them in the resting condition. A clear account of the presence of micronuclei in the quiescent state was first given by Maupas ('83, p. 661). More recently, Gruber ('86) has confirmed Maupas' announcement of their presence in *S. cæruleus*. Plate ('86), on the contrary, maintains that the minute bodies in question are "Assimilationsprodukte," basing his opinion on the fact that the "granules" are not visible in all specimens. As I have succeeded in finding them in mitotic division there is no longer reason for doubting their micronuclear nature.

The micronuclei of *Stentor* are difficult to find, not so much on account of their minuteness, as by reason of the vastly

¹ An interesting case of synchronous constriction of a large number of meganuclei is given by Gruber ('84b) for *Spirostomum lanceolatum*, n. s.

greater bulk of the deeply-staining meganucleus. It is useless to search for them until the Stentor has been cut into thin sections or the meganucleus isolated. The serial-section method is the most satisfactory, and the best stains I have tried are Czokor's alum cochineal and borax carmine. The micronuclei always lie close to the meganucleus, as usual among Infusoria (Figs. 19, *mcn.*, 25), but not always absolutely in contact with it. While sometimes seen with great distinctness, I have not been able to make them out in many specimens, although prepared in the same manner. They are peculiarly difficult to find in individuals that have entered upon fission, owing to the fact that they swell and become almost unstainable at time of their division.

The number of micronuclei in Stentor is larger than in any other Infusoria, so far as known, and has been greatly understated by Maupas ('83, p. 661), who mentions as the highest number, 28 for *S. roeselii*. In serial sections of a Blue Stentor in which the micronuclei were unusually distinct, I have counted 66, and in another instance, 54. The absence of numerical agreement which Maupas ('83, p. 661) found the micronuclei of *Spirostomum ambiguum* to possess, with reference to the nodes of the meganucleus, also prevails in Stentor. While often only one micronucleus may be found adherent to a node (Fig. 19, *mcn.*), there are sometimes as many as seven or eight.

The micronuclei of *S. cæruleus* do not differ materially from those of other Infusoria. They are spherical, highly-refractive, deeply-staining bodies measuring 1.5μ – 2μ in diameter, apparently homogeneous and composed wholly of chromatin (Fig. 55). They are so minute and dense that I have been unable to discern a nuclear membrane in the resting state, but one is visible when the micronuclei are enlarged at time of fission or conjugation (Figs. 48, 56).

Hitherto, micronuclei have not been observed in the Stentors having simple meganuclei. I have found them in *S. igneus*, adherent in considerable numbers to the meganuclei (Fig. 25), and also in *S. pyriformis*. They are even smaller than the micronuclei of the Blue Stentor, measuring only 1μ in diameter.

The double nuclear apparatus of *Stentor* probably expresses the extreme of differentiation between micro- and meganuclei. The latter are highly modified as to structure and, as regards the higher *Stentors*, as to form; the former are simplified as much as possible, and reduced to a size so minute that, even with the highest powers, they appear as hardly more than mere specks lying on the surface of the immensely greater meganucleus.

Generalia.—There are few phenomena in cytology more interesting than the apportionment of nuclear functions in the Infusoria to two kinds of nuclei differing in size, structure, and mode of division. The meganucleus is comparable to certain somatic nuclei of the Metazoa that have become highly specialized to subserve some particular function; *e. g.*, the “giant nuclei” of gland- and excretory cells, found mainly among the Arthropods. The likeness between the two, as shown by the “massive,” granular structure, proneness to assume unusual shapes, and amitotic division, are very striking, and strongly suggest a similarity of function. The micronucleus corresponds to a metazoan germ-nucleus, and is the bearer of the “immortal” germ-plasm. The Infusorian, then, contains within the scope of a single cell both somatic and germinal elements. Through the exchange and copulation of micronuclei at time of conjugation, the perpetuity of the somatic part is assured in much the same way that the endless new generations of a Metazoan, each with its wonderful somatic development, are made possible by the periodic activity of the germ-plasm, likewise brought into play by the union of a male and female pronucleus.

B. FISSION.

The important process of self-division, or fission, has received more attention in the genus *Stentor* than in any other group of Infusoria. We have, in the first place, the surprisingly clear and accurate account by Abraham Trembley;¹ we have the descriptions and figures by Ehrenberg ('38), Balbiani ('60), Stein ('67), Moxon ('69), Cox ('76), and Schuberg ('90). The

¹ Phil. Trans. Roy. Soc. xliii, No. 474, p. 180, 1744.

external or cytoplasmic phenomena have been studied more frequently and are more accurately known than the nuclear changes, our knowledge of the latter being wholly due to the researches of Stein and Balbiani, made long before the discovery of modern cytological methods.

It was not until I had made considerable progress in the study of the fission of *S. cæruleus*, that I had access to Schuberg's account of it. I continued the work in order to test his results by personal observation, and perchance add something to his important contribution to the subject. I have, furthermore, thought it desirable to publish a series of drawings (Pl. XXIV, Figs. 26-37) illustrating the stages of fission of *S. cæruleus*; for hitherto no complete and accurate series has been given for any species of Stentor. These figures obviously do not represent successive stages in the bipartition of one individual; for that, by reason of the occasional change of position and contraction of the animal, I have found impracticable.

I. *Cytoplasmic Phases.*

The first sign of fission is the formation of a rift (the anlage of the new adoral zone) in the pellicula and ectoplasm near to and almost parallel with the left boundary-stripe of the ramifying zone. (Fig. 26, *a.z.*¹) This cleft has an early longitudinal direction; yet, as Schuberg has already shown, it cuts across the stripes of the ramifying zone, forming narrow angles with them (Fig. 26). Schuberg represents the anlage of the new zone as being very short at the outset, and gradually extending at the extremities, which curve more and more toward the right. This very early stage appears to be extremely transient, for out of the many specimens I have studied in the first stages of fission, I have seen but one or two that had a short rift.

The rift opens at the outset to the full width of the adoral zone. It opens entirely through the ectoplasm, as shown by the absence of pigment, so that the soft endoplasm is exposed and protrudes a little. From the start it exhibits a ciliary motion. At the very beginning this movement seems as undefined as that of the protruding cytoplasm of the foot;

but gradually, as the young membranellæ develop from the exposed endoplasmic ridge, the motion is restricted to undulations sweeping lengthwise along the new adoral zone. (Figs. 26, 27, *a.s.*¹) The wave-like motion becomes more and more pronounced as the membranellæ increase in length and vigor of action (Figs. 30, 33, 35-37). It continues throughout all subsequent stages of fission, and even for a short time after division is accomplished. It is but rarely seen in old membranellæ, the action of which is more rapid and uniform.

The posterior extremity of the new zone, reaching about two-fifths of the way down the body when the Stentor is extended, becomes more sharply curved than the anterior end. Just within the curve the pharyngeal invagination appears (Figs. 27, 28, *o.*¹). At this and the preceding stages, the aspect of the new zone is different according as the animal is in the extended or contracted state. When contracted the zone forms nearly a semicircle, and has strongly incurved extremities (Fig. 28). The new membranellæ are quiescent, and the whole zone is apt to stand out in strong relief upon the surface of the animal.

A structure of much morphological interest which makes its appearance very early, is a narrow light band running parallel with the zone along its right side (Figs. 27, 30, 32, *p.*¹), and having much the appearance of a clear stripe of unusual width. It persists throughout the phases of division, and is seen in the fully-formed frontal field, which it encircles just within the adoral zone (Figs. 6, 30, *p.*), in the manner described by Schuberg. His interpretation of this band as a rudiment of the peristome of the lower Heterotricha, seems the most reasonable explanation. It is certain, as Schuberg (p. 234) maintains, that the frontal field ("Stirnfeld") of Stentor and its near allies, Climacostomum and Folliculina, cannot be regarded as homologous with the true peristome of the lower Heterotricha. Neither the structure of the frontal field nor its mode of formation are the same as those of the peristome. As Schuberg has pointed out, the frontal field of Stentor is a piece of the ventral body-wall, cut out, as it were, by the adoral zone during fission, finally shifted to a horizontal posi-

tion at the anterior end of the posterior zoöid, and almost encircled by the adoral zone. The peristome, if it persist at all, must remain adjacent to the adoral zone on its inner side (right side during the earlier stages of fission, in which it occupies exactly the same place as in *Spirostomum*), and, therefore, in the precise position of the light band.

The area of body-surface partially enclosed by the adoral zone and destined to become the frontal field of the posterior individual, undergoes little change up to the time of the formation of the pharynx (Fig. 27, *f*¹). At this stage its stripes, already narrower than those outside the ramifying zone, become very much reduced in width, presumably by intercalation of new "clear stripes" (Fig. 28, *f*¹); but this I have not been able to prove by direct observation. As fission advances, the stripes of the new frontal field become narrower and more numerous, thus approaching the condition of the old frontal field (Figs. 33, 35, 36, *f*¹). The relative width of the frontal stripes to those at same plane is clearly shown in a transverse section (Fig. 8, *f*¹).

The phases thus far described, although preliminary to the actual fission, are extremely important, including as they do the principal *formative* act of the process—the development of a new adoral zone. The gradual evolution of structures so complicated as membranellæ from a mass of indifferent protoplasm, is very striking. The fact that the membranellæ are derived from the outer layer of the endoplasm is not without interest, showing as it does an ontogenetic development different from the phylogenetic. For there is every reason to believe that the membranellæ were originally formed from cilia or ciliary structures belonging to the ectoplasm. But the ectoplasm is itself a modified layer, and hence not so well fitted to give rise to a new and complex structure as the undifferentiated endoplasm. It may further be noted that its thickness is hardly sufficient to furnish material enough to build up the membranellæ.

Another organ that is formed *de novo* very early in fission is the contractile vacuole (Fig. 26, *c.v.*¹). At first it is always smaller than the old contractile vesicle. In *S. cæruleus* it

evidently originates from one of the vacuoles which commonly lie in a row posterior to the contractile vesicle and often extend into the peduncle. At a point always a little above the level of the posterior extremity of the new zone, one of these vesicles takes on the contractile function and at once acquires the excretory pores (Fig. 26, *ex. p.*) originally described by Moxon ('69). It contracts at regular intervals, but not synchronously with the old vacuole.

The formation of the new vacuole in *S. roeselii* is brought about by a local dilatation of the longitudinal canal. It seems to have been first observed by Balbiani ('81, p. 322), but was studied independently by me while still in ignorance of Balbiani's mention of it. The dilatation of the canal occurs early in fission, preceding the formation of the mouth, and occupies the same relative position as the new contractile vacuole in *S. cæruleus* (Fig. 29, *c.v.*¹). The new vacuole retains its connection with the portion of the longitudinal canal posterior to it, and for a time with the anterior portion also.

The further history of the excretory system of *S. roeselii* at time of fission offers a sufficient explanation of the much-discussed "ring-canal" discovered by Lachmann and figured in Claparède and Lachmann's great work on the Protozoa ('59). The "ring-canal," encircling the anterior end of the animal, just under the adoral zone, has been looked for in vain by nearly all modern micrographers. But Maupas ('83, p. 641) claims to have seen it in *S. cæruleus*, and Bütschli states ('89, p. 1443) that unpublished drawings of Engelmann (1861) show the ring-canal in *S. cæruleus* and *S. roeselii*.

The study of *S. roeselii* in fission gave me the true history of the ring-canal, as the diagrams in Fig. II will show. It is seen that the ring-canal (*rc.*) is that portion of the longitudinal canal lying immediately anterior to the new contractile vacuole (*cv.*). During the earlier stages of fission (*a*) it runs straight forward to the old vacuole; but as the new zone curves round and takes a more nearly horizontal position, the adjacent portion of the longitudinal canal curves with it, and in doing so has to lengthen (*b*). The newly-formed ring-canal is finally severed from the more anterior portion of the longitu-

dinal canal by the separation of the daughter-stentors—or possibly somewhat earlier. Within a few hours after fission, the ring canal atrophies without leaving a trace. It is evident, then, that Lachmann saw the ring-canal in a young specimen of *S. roeselii*, and later observers have for the most part failed to find it because they did not obtain young specimens.

As soon as the new zone has reached its full length, has curved inward at the ends, and its membranellæ have attained

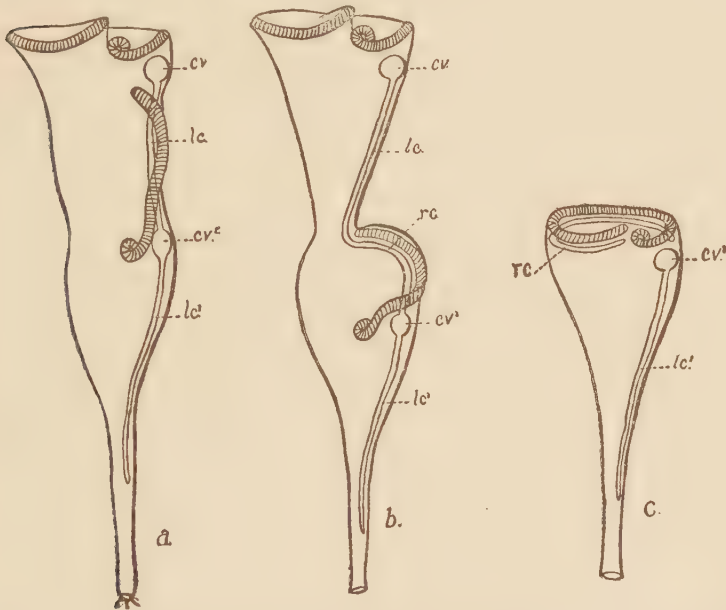


FIG. II. — Diagrams to illustrate the formation of the ring canal in *S. roeselii*. *cv.*, contractile vacuole; *cv.*¹, new contractile vacuole; *lc.*, *lc.*¹, longitudinal canal; *rc.*, ring-canal.

a definite shape, the new pharynx begins to develop (Figs. 27, 28, *o.*¹). It starts as a slight depression in the body of the Stentor just at the posterior tip of the new zone. The first direction taken is obliquely inward towards the right. The invagination becomes deeper and larger, then turns spirally and pushes directly into the endoplasm (Fig. 13 *o.*¹ *inv.*). The tip of the new zone follows the invagination *pari passu*, so closely as to give the impression that the zone is pushing into

the cytoplasm. The new velum and buccal pouch (Figs. 33, 36, *vel. 1*) become evident during the late stages of fission. The formation of the new pharynx produces no break in the integument, except at the inmost point where the mouth is located, and the pharynx is, therefore, lined with an integument that once covered the surface of the body; it is comparable to the stomodæum of a Vertebrate embryo.

As soon as the new pharynx begins to develop, the body of the Stentor becomes considerably elongated and more cylindrical than in the usual condition. A slight constriction appears about midway of its length (Fig. 27). This constriction is probably a mere result of the elongation, and has nothing to do with the fissional constriction which appears later. It seldom or never coincides with the fission-line (Fig. 31). Synchronous with the constriction above-mentioned, and perhaps a result of it, I have noticed a decided bend to the left in the new adoral zone (Fig. 30). This bend is important as indicating the point at which the fission-line starts (Fig. 31, *l*). The formation of this cleavage line was first observed by Moxon ('69), and has been accurately described by Schuberg (p. 228). I have little to add to his account. Its advent is so sudden, and it extends so rapidly around the animal, that it is difficult to ascertain where it first appears. Schuberg states that it starts on the left border of the new zone at the point where the latter reaches the left boundary-stripe of the ramifying zone. I am at least satisfied that this is the first point where the fission-line touches the zone. Thence it passes to the right around the body, cutting all the stripes at right angles (Fig. 31, *l*) until it reaches the right side, where it extends obliquely upward to meet the curved aboral end of the new zone. I do not find that it approaches the new mouth so closely as Schuberg has represented, but rather takes the direction shown in Fig. 33. At a slightly later stage the starting-point of the fission-line is found to have passed above the anterior curve of the new zone, now very pronounced, and to have joined the other extremity (Fig. 22, *l*). The process by which the transposition is brought about I have not been able to follow. The constriction which at length separates the two

zoöids now takes place in the path marked out by the fission-line. I cannot agree with Schuberg's view that the constriction is accompanied by a rupture of the pellicula. He says: "Bei Stentor nun muss . . . ein Durchreissen schon früher [than in other Infusoria] stattfinden, oder mit einem Wort *die ganze Durchschnürung ist von Anfang an mit einem Reißen der Pellicula* in bestimmter Richtung verbunden." Now, any rupture of the pellicula occasions a protrusion of the cytoplasm whenever the animal contracts, notwithstanding the strong tendency of the integument to close over every wound. Since no protrusion of cytoplasm is visible along the line of fission, it seems very doubtful whether the pellicula is actually ruptured. Longitudinal sections of Stentors in the middle and later stages of fission show no evidence of a rift in the pellicula.

Although I do not consider it a rupture of the pellicula, I have not come to a satisfactory conclusion in regard to the nature of the fission-line. It certainly divides the substance of the blue stripes (ectoplasm), and so produces a white line around the body, not unlike one of the clear stripes. Sections show that the line is superficial, and not the optical expression of a plane passing through the animal, as in ordinary cell-division. I have sometimes thought that a contractile fibre of extreme fineness was developed in close connection with the pellicula, which, by contracting, brought about the constriction in the manner of a string passed around a yielding body and tightened. The supposed fibre, however, may be merely the sharp fold in the integument, caused by constriction.

From the study of preparations I feel confident that the myonemes are not cut by the fission-line, but simply become bent sharply inward (Fig. 34). Until a late stage of fission the stripes on either side of the line remain very accurately matched (Figs. 31-35), notwithstanding the notable narrowing of the stripes at the posterior end of the distal individual.

At this point a series of remarkable changes in the position of the new frontal field and adoral zone begin, and proceed rapidly until the completion of fission. Hitherto the plane of the new frontal field has remained nearly parallel to the surface of the Stentor. It now begins to project more and

more, and gradually approach the horizontal position (Fig. 33, *f*¹). This is accomplished by two distinct processes, — a shoving out of the cytoplasm towards the left, and a depression of the aboral end of the new zone, combined with a strong curvature dorsalward. The backward movement of the zone is brought about by a deep, narrow constriction between its projecting anterior end and the body of the Stentor (Figs. 32, 33). This is only a part of the general constriction which now obliquely encircles the animal coincident with the line of fission, but is much the deepest at this point. The contour of the new frontal field varies according as the animal is contracted or extended; when in the former condition it is concave (Figs. 31–33, the last being semi-contracted), whereas in the latter it is nearly flat.

Another factor in the process of fission is the elongation of the posterior portion of the distal zoöid. This starts at the beginning of constriction, and rapidly progresses *pari passu* with the constriction. Thus the distal zoöid speedily acquires the typical tapered form of Stentor (Figs. 35, 36). The elongation is to be referred to the same cytoplasmic action that produces ordinary extension. There are, then, three distinct factors in the middle and later stages of fission: (1) out-pushing of new zone into a horizontal position, (2) constriction, and (3) elongation of posterior part of distal zoöid. The shifting of the new zone into a horizontal position is accompanied by a shortening and broadening of the frontal field, and a more complete circumscription of it by the adoral zone (Figs. 35–37). Throughout the middle and later stages of fission the distance from the new pharynx to the fission-line remains nearly constant, but towards the end, when the constriction has become very great, this space is materially reduced, although, even in the newly-formed proximal individual, the unenclosed space on the ventral margin of the frontal field is considerably greater than in the ordinary condition. The reduction of the unenclosed space is brought about by the upward movement of the oral spiral, which is the last portion of the adoral zone to take a horizontal position.¹

¹ This is more clearly seen in Fig. 46 *g*, Pl. XXV, than in Figs. 35, 36, because in the latter the zone is viewed obliquely.

The changes in the stripes during fission are an index to the extraordinary alterations in the contours of the animal. The most obvious modifications are in width and length. The granular stripes of the new frontal field are about half the breadth of those of the ramifying zone from which they are derived ; while the granular stripes at the posterior end of the distal zoöid become at length scarce a fifth of their former breadth. Their attenuation has kept pace with the constriction, and what they have lost in breadth they have gained in length. That the stripes are viscous like the rest of the cytoplasm, and have been drawn down to greater fineness by the elongation of the portion they cover, seems to me to explain sufficiently their attenuation. Another explanation must be found for the narrowing of stripes in the new frontal field ; for here certainly no lengthening of stripes takes place. I have postulated an increase in the number of striæ through the bisection of each granular stripe by a clear stripe. Schuberg (p. 227), although taking note of the increase in superficial extent of the new frontal field, nevertheless says : "Eine Vermehrung der Körperstreifen innerhalb derselben [*i. e.*, the frontal field] hat dabei ebensowenig wie bisher statt. . . . Die Zahl der Streifen, welche durch das Wachsthum der neuen Zone nach rechts und links im Ganzen durchquert wurden, beträgt zwischen 30-40, was denjenigen auf dem Peristom ausgebildeter Thiere entspricht." Now, as the number of granular stripes in the ramifying zone varies, according to Schuberg, from 20 to 40, the number of stripes in the new frontal field must also be variable, and nothing short of counting in the living animal the number of stripes enclosed at the outset by the new zone, and again counting them in the fully-elaborated frontal field, and finding the numbers to agree, would warrant Schuberg's statement. I admit that my own view of the reduplication of stripes is based—not upon an actual count, for I have found that impossible—but upon their much greater fineness in the fully-formed frontal field.

The formation of the new ramifying zones is worthy of notice. That of the proximal zoöid is nothing more than the posterior portion of the original ramifying zone, as will be seen

by a comparison of Figs. 26-28, 33, 35-37, *r.z.* The ramifying zone of the distal zoöid is, on the contrary, formed anew. As soon as the constriction at the anterior end of the new zone has cut down an appreciable distance along the side of the animal, a very distinct derangement of stripes appears immediately above it, and lengthens with that portion of the distal zoöid (Figs. 35, 36, *r.z.*¹). The newly formed ramifying zone is different from that of older individuals, inasmuch as it is not strictly a branching, but an inosculation of stripes, meeting at an angle of about 45° when the animal is extended. This condition of the ramifying zone persists for some hours after fission.

How is the new ramifying zone produced? Schuberg explains it as a result of a rupture of the pellicula in the fission-line, and apparent weight is lent to this view from the fact that lesions almost invariably lead to disorganization of the stripes as a result of the closure of the wound. A rupture of the pellicula, however, is not necessary to account for the ramifying zone. An inspection of Figs. 32 and 33 will show that the constriction-cleft at the aboral extremity of the new zone is the apex of the fission-line, which on both dorsal and ventral sides cuts the stripes obliquely. As the constriction becomes deeper, the ends of the stripes immediately posterior to the cleft in Fig. 33 must become pinched together as they come down to the constriction-line upon both dorsal and ventral sides of the anterior zoöid. This process continues until the end of fission. The result is a seam in the side of the distal zoöid, where the stripes meet and inosculate at their tips as above described.

After the daughter-stentors have assumed nearly their definitive shape, separation is effected by rupture of the connecting thread at the point where it joins the proximal individual. This is accomplished partly by the pulling and torsion of the distal individual, and in part by the sudden in-cutting of the now much reduced line of constriction (Figs. 36, 37). The latter process brings about a speedy separation (in most cases) of the twin zoöids, and also leaves an area of naked cytoplasm for the foot (Fig. 37, *ps.*¹). Sometimes, especially if the animal is well-fed and vigorous, the incision separates the

twin Stentors at once, but more often they remain connected for some time by a thin cytoplasmic thread from the truncated tip of the anterior Stentor. (Fig. 37.) The point of attachment to the proximal zoöid is always at the aboral extremity (*d.az.*¹) of the adoral zone. The body stripes of the posterior zoöid are strongly curved towards this point, and this character, combined with the notable roundness of the body-portion of this zoöid, as compared with the distal one (Fig. 36) serves to distinguish the daughter-stentors for an hour or two after fission is completed.

The time consumed by fission in Stentor doubtless varies in the different species, although I have found it approximately the same in *S. cæruleus* and *S. polymorphus*. It has been variously stated. Stein ('67) was unable to give its duration, but was correct in saying that the early stages require more time than the later. Cox ('76) stated the period as only two hours for *S. polymorphus*—an understatement due to the fact that he did not see the slow early development of the new zone. Schuberg ('90, p. 225) estimated it at six and one-half hours for *S. cæruleus*, four hours being consumed in the early formative stages, and two and one-half in the actual fission. I have found that the duration of the process in *S. cæruleus* varies somewhat according to the vigor of the animal, and doubtless also according to the temperature, but my observation of it at 17°–20° C. agrees very closely with Schuberg's. I have repeatedly observed a period of about seven hours, but it is sometimes an hour or more less.

It is seen from the foregoing description that the cytoplasmic phases of bipartition are divisible into two periods, the *formative period*, in which the anlagen of new organula are laid down, and the *constrictional period*, during which the actual fission takes place. As we shall see later in the consideration of Regeneration in Stentor, it is in all probability the constriction and not the neoformation of organula, that determines whether fission shall take place, or merely renewal of mouth and adoral zone. Our ignorance of the *primum movens* to a neoformation is complete. We can only say it lies in some peculiar molecular condition that incites the duplication of existing organs. And

the working-out of the impulse thus given is only partially dependent upon temperature, food, the size of the individual, or even, as Balbiani's ('92) and my own experiments in merotomy (see p. 550) show, upon the intact condition of the organism.

2. Nuclear Phases.

Meganucleus.—The best observations hitherto upon the meganucleus at time of fission are those of Balbiani ('60) and of Stein ('67), later observers of fission in *Stentor* having paid little or no attention to the nucleus.

The most satisfactory method of studying the meganuclear changes is to select a specimen at an early stage of fission in which the meganucleus is clearly visible, and then watch the nucleus throughout its changes until the two daughter-nuclei have attained their definitive shape. This method I have supplemented and controlled by the examination of stained preparations, but these can by no means supplant the study of the living meganucleus.

At the beginning of fission the meganucleus has its usual spiral disposition in the body. The first alteration, just previous to the formation of the new pharynx, is a straightening of the nucleus and disappearance of the commissures, the nodes becoming appressed. (Fig. 26, *mgn.*; Fig. 47 *a.*) The next step is coalescence of the nodes into a solid mass, which shortens rapidly until it assumes a nearly spherical form (Fig. 41 *a.*). The time required for coalescence of the nodes is about one hour, but it varies in different individuals. Usually the meganucleus has assumed the spherical shape when the pharyngeal funnel has begun to form. Its shape at this time is rarely a perfect sphere and often it is jagged and irregular. In fact, when observed in the living state it is seen to be a plastic, fluctuating mass, almost amoeboid in its rapid change of form. (Figs. 46 *c*, 47 *d*, *e.*) These changes of form are the outward expression of an internal commotion that may be considered as a continuation of the forces that produced the coalescence of the nodes. In a few minutes the movements take a definite direction, producing an elongation of the

nucleus, always parallel to the long axis of the animal (Fig. 41 *d, e, f*). As soon as elongation has begun, a constriction appears about the nucleus, and persists for about half-an-hour (Fig. 41 *b-e*). In less than an hour the nucleus attains a slender, rod-like form (*f, g, h*), normally straight and cylindrical, but almost invariably bent or curved by the occasional contractions of the animal, as seen in Fig. 41 *g, h, i*. The meganucleus, however, always tends to straighten, and will do so if the intervals between contractions be sufficiently long.

When elongation has reached its limit, and sometimes perhaps even before, nodes appear at the tips of the meganucleus (Figs. 35, *mgn.*; 41 *i*). I have found no exception to the rule that the nodes develop first at the ends of the meganucleus. The nodulation advances rapidly and at nearly equal rate towards the middle of the nucleus, so that at any stage in the process one counts about as many nodes in one moiety of the nucleus as in the other; but there is usually a difference of one or two (Figs. 35, 41). The nodulation soon reaches the middle point of the nucleus, which normally lies in the plane of the constriction between the now nearly-divided daughter stentors. The newly-formed nodes are, as a rule, beautifully symmetrical and alike in size. As soon as the nucleus has become fully-noded, the middle commissure, lying just opposite the fission-line (Fig. 42) at once becomes longer and finer than any of the others. It differs from all the other commissures in being a commissure of division, comparable to the connective uniting the moieties of an amitotic nucleus. Rupture of the commissure takes place shortly before the separation of the daughter-stentors (Fig. 43), and the "tail-ends" at the adjoining tips of the meganuclei are speedily drawn into the terminal nodes.

The way in which nodulation takes place is worthy of note. The first thing observable is a series of constrictions, all of nearly equal depth and evenly spaced. At this early stage a stained preparation, such as Fig. 44 represents, shows that the chromatic substance has become aggregated in the incipient nodes, and the commissures are nearly destitute of it. Thus at the outset the commissures are relegated to the comparatively

unimportant duty of serving as connectives for the nodes. The rate of constriction at the different commissures is by no means uniform in the later stages of the process, and sometimes alternate commissures become developed sooner than the intermediate ones (Fig. 45), thus producing double nodes, which are of very frequent occurrence even in adult Stentors.

The mode of meganuclear division above described is undoubtedly typical for all Stentors having a moniliform or rod-shaped nucleus, and, indeed, for such meganuclei wherever they occur. It is, however, by no means essential that the actual division should be achieved in just this manner, although the process is always upon the same general plan: condensation, elongation, renodulation. The variation lies in the time at which division is interposed, and in this respect I have noted the following cases:

(1) Division may be deferred until the close of the phases, as in the foregoing description.

(2) It may occur at time of maximum coalescence, (Fig. 46 *a, b*).

(3) It may take place during the coalescence of the nodes. (Fig. 47, *a-d*.)

(4) The nucleus may be in two equal or nearly equal parts previous to fission, and no division whatever take place. But if the parts are very unequal (as in Fig. 35) division will occur as in (1) or (2).

Fig. 46, *a-g* represents a series drawn from the living nucleus, showing the phases when the nucleus divides at the moment of greatest condensation. The division was obviously unequal. Immediately after division both parts began to elongate, and soon came into contact (*d*). The larger, posterior piece lengthened more rapidly than the smaller, anterior portion. When the two daughter-nuclei pressed closely together at the point of contact, even bulging at that point owing to mutual pressure, I watched carefully to see whether fusion would take place. But it occurred neither in this instance nor any other in which I followed the process in the living meganucleus; they finally drew apart always at the very point where they had been in contact. Coalescence was evidently prevented by

the nuclear membranes. When both moieties had become moniliform they began to draw apart, the anterior remaining in the distal zoöid, the posterior lying mainly in the proximal, but about a fifth of its length extending into the distal offspring. (Fig. 46 *g*.) I have found it generally the case that when the meganucleus has divided somewhat unequally, the longer portion projects beyond the line of constriction into the zoöid having the shorter portion.

An interesting instance of division during coalescence is represented in the series of Fig. 47 *a-h*. The nucleus had originally 16 nodes. Fusion began at three different points: anterior, middle, and posterior (*a*), and proceeded slowly, requiring more than an hour to reach stage *d*. The result of the presence of three foci of coalescence was the attraction of material in opposite directions at two points, where consequently thin commissures were formed, which soon ruptured, thus breaking the nucleus into three pieces of unequal size (Fig. 47 *b, c, d*), of which the anterior contained the substance of 8 nodes, the middle of 6 nodes, the posterior of 2 nodes. Each piece soon attained its maximum coalescence, with repeated change of form (*d, e*), then the two anterior began to elongate, but the hindmost and smallest remained spherical until nodulation of the others had begun (*f, g, h*). The anterior piece, from which 12 nodes were formed, was apportioned to the distal individual; the other two, which together gave rise to 8 nodes (making 20 in all from a nucleus previously possessing 16), to the proximal. Thus the posterior zoöid started off in life with two separate meganuclei.

The fourth case, with absence of nuclear division, I have not actually observed, but that it occurs is evident from the considerable number of Stentors that possess two distinct meganuclei of about equal size.¹

¹ It is not easy to find a satisfactory explanation of the variation in the time at which meganuclear division takes place. Such a variation has not, so far as I know, been observed in other species of Infusoria; but negative evidence on this point is of little value, considering the small number of recorded observations. The variation in *S. cæruleus* is apparently limited to the three periods above stated; I have never seen division during the period of elongation (Fig. 41 *c-h*). I am inclined to regard the division at time of condensation (Fig. 46 *a, b*) as the

The remarkable metamorphosis undergone by moniliform and rod-shaped meganuclei at time of fission appears to be purely a change of form. As stated on a preceding page, I have not been able to detect the least structural alteration in the substance of the meganucleus at any stage. Perhaps the most obvious explanation of the changes is, that an equal division of the meganucleus in its moniliform, or cord-like, flexuous state is not easily brought about, owing to its spiral disposition in the body and the frequently unequal size of its nodes. The case is different when the nucleus divides after having returned to the moniliform condition, for the nucleus is then nearly straight, its middle point coincides with the plane of division, and the nodes, newly formed from a cylindrical rod of even diameter from end to end, are very nearly alike in size. That the division is fairly equal is evinced by the fact that the number of nodes in each daughter-stentor is usually about the same, as the following table will indicate:—

NO. OF PAIR.	NODES.	NO. OF PAIR.	NODES.
1	12—12	12	10—11
2	8—8	13	12—13
3	15—15	14	15—16
4	10—10	15	14—15
5	10—10	16	14—12
6	11—11	17	14—16
7	13—13	18	15—13
8	12—12	19	12—8
9	11—11	20	19—15
10	9—9	21	14—18
11	14—14	22	17—12

It is seen that in eleven instances out of the twenty-two given the number of nodes in each daughter-nucleus is exactly the same; in four other cases there is a difference of only one node. In 68 per cent, then, of the specimens examined, the division is fairly equal. But how shall we account for the four instances (pairs 19–22) of decidedly unequal nuclei? I believe

primitive type—a reminiscence of the time when the nucleus was always spherical. The transient appearance of a constriction about the nucleus (Fig. 41 *b-c*), whether it divides at this time or later, lends strength to this view; for on this basis we must regard it as an inherited tendency to division.

that they are due to premature, unequal divisions, such as I have described in detail (p. 515). This was certainly the case with pairs 19 and 20, the latter being represented at an early stage of nodulation in Fig. 35, and the former being shown in detail in Fig. 47.

Another reason for the reconstruction of the meganucleus is that it provides a means of increasing the number of nodes ; for increase does not, so far as my observation goes, take place while the nucleus is in its resting form. Increase of nodes is beautifully displayed in those species where the number of nodes is constant (*e.g.* in *Stylonichia*, *Onychodromus*, and many other *Hypotricha*), for in these forms the number is exactly doubled after condensation (Balbiani '60, Bütschli '76). In *S. cæruleus*, contrary to the statement made by Balbiani in 1860 (p. 77), and re-affirmed by him more recently ('81, p. 325), the number of nodes is not regularly increased to double the number in the parent ; yet an increase almost invariably takes place after condensation.¹ Thus the offspring are furnished with nuclei each having a number of nodes considerably greater than half the number possessed by the parent. The nodes are of course smaller.

Micronuclei. — The division of the micronuclei of *Stentor* at time of fission has not hitherto been described. By means of sections of *S. cæruleus* I have seen micronuclei in mitosis (Fig. 48), but in only a single specimen, although a large number were sectioned in all stages of fission. There is considerable difficulty in making out the micronuclei at all during fission, because they become very transparent and of nearly the same refractive index as the surrounding cytoplasm. Previous to formation of the spindle, the micronuclei enlarge from the normal size of 2μ to a diameter of $4-5\mu$; at the same time they lose most of their stainability and their high refractive power. The spindles represented in Fig. 48 are evidently at different stages ; the one on the right shows the

¹ I have made only a few observations on this point. In one instance a *Stentor* in the first stages of fission had a nucleus of 17 nodes; the offspring had nuclei of 14 and 15 nodes respectively (pair 15, p. 516). Another case — increase from 16 nodes to 20 — has already been mentioned (p. 515).

"equatorial plate" distinctly, while that on the left is at the "spirem" stage figured by Maupas ('89, Pl. IX, Figs. 7-9). The meganucleus, of which only a peripheral section appears in the drawing, was at complete condensation, and in two distinct pieces. I made out 65 micronuclei adherent to the two, but none were found in the spindle stage except the two above-mentioned. It would be of interest to know whether all the micronuclei divide at every fission, and an even allotment of them to each daughter individual takes place.

A point worthy of note is the time at which micronuclear division takes place in different species of Infusoria, with reference to the phases of the meganucleus. In *Stentor* it is considerably earlier than in *Paramecium*, *Stylonichia*, or *Vorticella*, where the division of mega- and micronuclei are nearly simultaneous.

3. *Ontogeny and Phylogeny.*

The conception that the development of a new Infusorian by the process of fission is an ontogenetic development, comparable in some respects to the development of a Metazoön, has impressed itself strongly upon me in the study of fission in *Stentor*. I do not regard the formation of the proximal offspring as homologous with the building-up of a pluricellular animal from the egg. It is rather to be compared to the agamic reproduction of Metazoa by strobilation or segmentation, as in the *Scyphistoma* of *Acalephs*, in the *Turbellaria* (*Microstomum*), and in *Annelids* (*Autolytus*, *Nais*, etc.); for a somatic portion of the parent is converted by the regeneration of necessary organs into a new being, whereas the development from the egg has a single germ-cell as its starting-point.

Is it possible to discover in the stages of fission a repetition of the leading facts in the phylogeny of the species? When we consider how doubtfully and indistinctly phylogenetic history is generally expressed, even in the ontogeny of Metazoa, we shall not be surprised to find the evidences of such recapitulatory stages in the evolution of a Protozoön far from decisive. And while in most groups of the Metazoa we have two checks upon our embryological inferences regarding

phylogeny — comparative anatomy and palæontology — among the Infusoria we have only the former.

I regard the Heterotricha as showing more clearly than any other group of Infusoria a phylogenetic history. They possess one structure, the adoral zone, that we can consider absolutely homologous from the highest to the lowest of the group. Another structure, the peristome, which lies to the right of the zone and in the higher forms is partly or almost wholly enclosed by it, is also probably homologous throughout all the lower forms of the group. The fact has already been noted (p. 502) that in the Stentorina the peristome is replaced functionally by the frontal field. These three structures, adoral zone, peristome, and its successor, the frontal field, are more important than any others in studying the comparative anatomy of these forms. In passing from the lower to the higher Heterotricha, we find that the peristome and zone take a more and more terminal position, until in the Stentorina (*Climacostomum*, *Stentor*, *Folliculina*) it is completely apical (Fig. III, *d, e, f*). It is possible, then, to arrange a series (which we may regard as phylogenetic) based upon the position and development of the adoral zone and frontal field (Fig. III, *a-f*). In this series the forms in which these structures are most strictly lateral in position (*Spirostomum*, *Blepharisma*) will occupy the lowest place, and the others (*Condyllostoma*, *Climacostomum*, *Stentor*) be arranged according as the zone becomes more and more terminal, and as the peristome (or its physiological equivalent, the frontal field) increases in breadth and becomes more and more circumscribed by the zone.

This much being attainable by comparative anatomy, does the ontogeny of one of the higher forms (*e.g.* *Stentor*) yield any evidence for or against the arrangement given in Fig. III? In comparing the various stages in the evolution of the zone and frontal field of the proximal zoöid of *S. cæruleus* (Pl. XXIV, Figs. 26-36) with the series of lower forms (Fig. III, *a-d*), we see that the successive positions taken by the new organula in question correspond quite accurately with the phylogenetic development of these structures. An exception is seen in the case of the peristome. This appears as a narrow band along

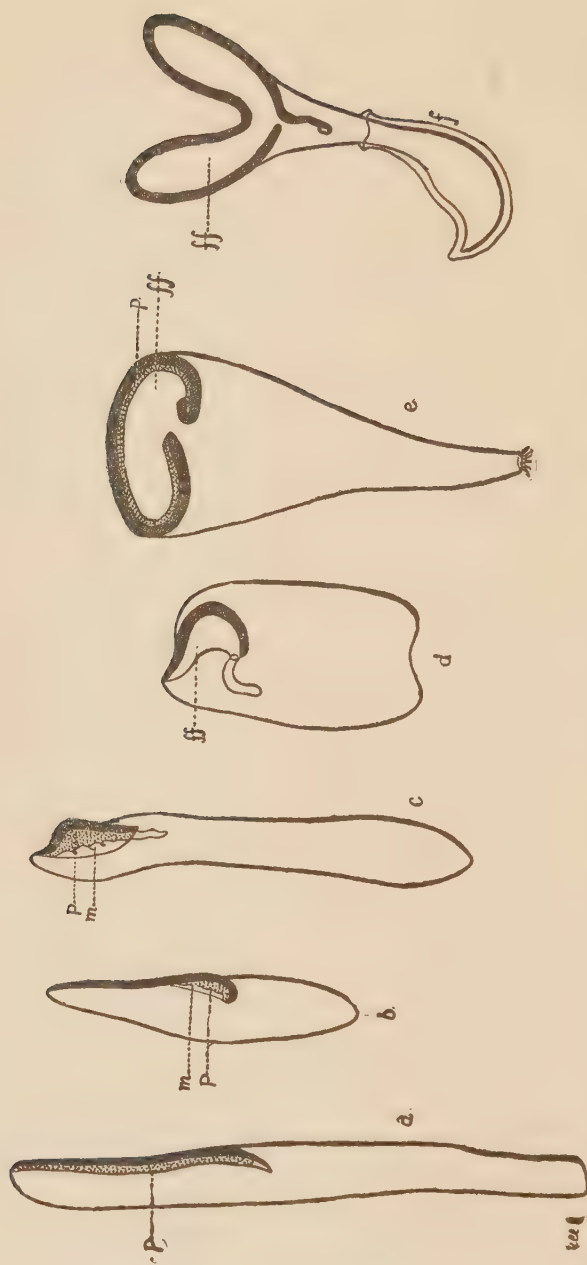


FIG. III.—Diagrams to illustrate the phylogeny of the Heterotricha. *a*, Spirostomum; *b*, Blepharisma; *c*, Condyllostoma; *d*, Climacostomum; *e*, Stentor; *f*, Folliculina. *p*, Peristome; *m*, undulating membrane; *ff*, frontal field. The heavy black line indicates the adoral zone.

the right side of the zone, and a narrow band it remains throughout. It is a case of a rudimentary organ persisting after its function has been assumed by another structure.

The evolution of the zone and frontal field does not end with *Stentor*, although in this genus these structures are completely terminal. In *Folliculina* the field is produced into two lateral, ear-like appendages. (Fig. III, *f*.) Unfortunately, we lack sufficient information regarding the evolution of the new zone in *Folliculina* to determine whether it has a *Stentor*-like stage or not; but the brief account of the fission by Möbius ('86) shows that it is very aberrant, and obviously adjusted to the peculiar mode of life and highly-modified frontal field of this form. It is therefore improbable that it will reveal much in regard to the phylogeny of *Folliculina*.

C. REGENERATION.

One of the most remarkable events in the life-history of *Stentor* is the periodic renewal of the mouth, pharynx, and adjacent parts of frontal field and adoral zone. That Infusoria are able to regenerate missing organs after encystment, conjugation, or merotomy, is well known. The peculiarity as regards *Stentor* is that regeneration also occurs when the parts to be renewed are already present and functional.

Considering the frequency of the regenerative process in *S. cæruleus*, it is surprising that it so long escaped detection. Specimens in course of regeneration were indeed seen by Stein ('67) and Schuberg ('90). It remained, however, for Balbiani ('91^b) to discover the real character of the process, and follow it through its stages. My own study of *S. cæruleus* had included the regenerative phenomena, and I had seen every stage of it previous to the publication of Balbiani's paper. I am able, therefore, to confirm his results from wholly independent observation, and add besides a few new facts. At the outset, I regarded the process as an abortive fission, believing that under certain circumstances the animal was unable to bring division to a successful issue, and its new frontal field then coalesced with the old, thereby inducing the atrophy of duplicated parts. But observation of the fact that successful

fission may follow closely the supposed abortive fission without change of the conditions of life, and the inevitable occurrence of the process when the adoral zone has become defective through atrophy or injury, have led me to adopt Balbiani's explanation as the true one.

1. *Cytoplasmic Phases.*

At the outset, the neoformation leading to regeneration is indistinguishable from that leading to fission. In both cases a new adoral zone is initiated in precisely the same manner and place. The first mark of distinction is the appearance of a new contractile vesicle if it is to be fission, and none if regeneration. But my observations of the process agree so well with Balbiani's description that there is no occasion to give them at length. Different stages of the regeneration are shown in Figs. 38, 39 A, and 39 B. It is interesting to see how perfectly the new membranellæ (*a.z.*¹) become assimilated to those of the old zone. The *modus operandi* by which the new mouth (*o.*¹) is gradually brought into the position of the old, and the slow advancement of the new zone to the anterior end of the animal, I have not been able to comprehend, nor does Balbiani offer any explanation. There is evidently a shortening of both the old and new zones.

During their formation, and until the new mouth has advanced nearly to the position of the old, the new membranellæ exhibit the same slow, undulating vibration as in fission. This is gradually quickened into the more vigorous vibration of the old membranellæ.

I have once seen two successive regenerations coming at a very short interval—a sort of hypertrophy of the process. (Figs. 39 A, 39 B.) When first observed the animal was at a slightly earlier stage than that represented in Fig. 39 A. The process, so far as the first-formed mouth (*o.*¹) and zone (*a.z.*¹) were concerned, was taking place normally. The old mouth (*o*) had nearly atrophied. To my surprise a second regeneration was also under way, the adoral zone being already visible. It developed rapidly, while its predecessor as rapidly disappeared (Fig. 39 B). The condition represented in Fig.

39 A was reached at 12.30 P.M., and that of Fig. 39 B at 2 P.M. A fact worthy of notice is that as soon as the second new mouth (ϕ^2) was developed the first (ϕ^1) began to atrophy, and also the newly-formed membranellæ of $a.z.^1$ between the junction of $a.z.^2$ with it and the mouth. Points of difference between this double regeneration and an ordinary one were the well-marked places of junction between older and newer adoral zones, and the slowness with which the process was completed. Twenty-four hours after the stage shown in Fig. 39 B, the new mouth had not quite reached its normal place.

As if to show the close connection existing between regeneration and fission, a Stentor that has started to renew its oral organs may undergo fission instead. I have only once observed it. When first seen, the new zone had already joined the old. I noticed, however, that a new contractile vacuole had appeared in the same place as in fission. Presently I noticed that the most anterior membranellæ of the new zone were becoming smaller. They soon atrophied entirely for about one-fourth the length of the new zone, and only a streak on the pellicula indicated where the zone had previously extended. The meganuclear phases were watched, and have already been described (Fig. 47). The subsequent cytoplasmic changes proceeded normally. Within the new ramifying zone of the distal zoöid a curious derangement of the stripes was observed (Fig. 40), undoubtedly due to the atrophy of the anterior portion of the adoral zone.

The time required by the process of regeneration varies in different individuals and doubtless at different temperatures. At 16°–20° C. I have found it requires 6½ to 8 hours. It is, therefore, a little slower than fission.

2. Nuclear Phases.

Balbani ('91) calls attention to the interesting fact that the meganucleus goes through precisely the same changes in regeneration of the oral organs as in fission, with the exception that division normally occurs only in the latter instance. But I find him at error in the statement that the number of nodes is not increased at time of regeneration. I have made

repeated observations on this point, under conditions that admitted of accurate counting of the nodes both before and after regeneration, with what results the following table will show :—

STENTOR.	BEFORE REGENERATION.	AFTER.	STENTOR.	BEFORE REGENERATION.	AFTER.
1	9 nodes	15 nodes	10	15 nodes	13 nodes
2	11 "	13 "	11	12 "	13 "
3	16 "	16 "	12	12 "	16 "
4	7 "	16 "	13	19 "	14 "
5	13 "	13 "	14	9 "	17 "
6	20 "	22 "	15	13 "	19 "
7	12 "	13 "	16	10 "	15 "
8	19 "	20 "	17	11 "	16 "
9	18 "	20 "	18	15 "	17 "

Out of the 18 examples, 14, or nearly 78 per cent, show an increase of nodes. In two (Nos. 3 and 5) the number remains unaltered, and in two (Nos. 10 and 13) there is a decrease. Sometimes the nodes are more than doubled in number (No. 4). While the extent of the increase in the number of nodes is highly variable, it is as a rule greatest in meganuclei with a small number of nodes (Nos. 1, 4, 14, 16, 17), and smallest in meganuclei with an uncommonly large number (Nos. 6, 8, 9). The average number of nodes for the 18 regenerated Stentors is 16, while the average number for the 44 young Stentors (*i.e.* 22 pairs) given on p. 516 is only 12.6 nodes. It is possible, then, to see an important function of regeneration in the marked increase in the number of nodes over and above those formed at time of fission; for in this way the superficial extent of the nuclear substance is greatly enlarged. If the nodulation of the meganucleus has a physiological value—and we can hardly conceive of its being so carefully maintained unless it has—it is reasonable to suppose that an increase in the number of nodes, up to a certain limit, is for the advantage of the organism. We have seen that the process of renodulation brings about the same result, whether it occurs at time of fission or at time of regeneration. The increase of nodes in the former instance is for the advantage of the race, in the latter for the benefit of the individual.

The table shows that there is by no means always an increase of nodes; sometimes there is even a decrease. In regard to No. 13, there seems to be a reason for the great reduction in their number. The Stentor was very small, and the long nucleus, with its 19 nodes, almost filled the body. The frontal field and adoral zone were almost atrophied, and this circumstance was, unquestionably, the prime motive for regeneration. I believe that in this case it was an advantage to reduce the extent of the nuclear surface, and this was certainly accomplished by reducing the number of nodes.

Although I have sectioned many specimens in various stages of regeneration, I have not been so fortunate as to discover the behavior of the micronuclei. The same difficulty encountered in the study of them at time of fission was met with here—they become so transparent as almost always to escape observation. The question, Do they divide at time of regeneration? therefore remains unanswered. If such should prove to be the case, the extraordinary number of micronuclei in Stentor would be accounted for.

Doubtless the regeneration of buccal organs during the ordinary condition of life obtains in all species of Stentor, and may yet prove to be of wide prevalence among Infusoria. Single stages were seen by Stein ('67) in *S. caeruleus*, *S. polymorphus* and *S. niger*. Besides the Blue Stentor, I have studied it in *S. polymorphus*, where it is carried out in the same manner, and requires about the same length of time.

3. Generalia.

There are four—possibly five—occasions in the life-history of Stentor when a regeneration of the oral organs is imperative. They are:

1. Fission. New frontal field, adoral zone and pharynx formed *in toto* for proximal individual. No atrophy of existing organs.
2. After encystment. Not yet actually observed in Stentor, but undoubtedly occurs.
3. After amputation of, or serious mechanical injury to, the organs of nutrition. Often atrophy of defective parts, which are replaced by the newly-formed ones.

4. After enfeeblement or degeneration of organs of nutrition. Complete atrophy of old pharynx, and partial atrophy of old frontal field and zone.

5. After conjugation (?) Not yet observed in Stentor. Has been recorded in Spirostomum (Maupas, '89) and many other Infusoria (Maupas, R. Hertwig, '89).

On every occasion when regeneration is required, it is carried out in the same manner. In no instance have I followed the development of a new oral apparatus that did not entail meganuclear reconstitution. There is a subtle bond of union between the two phenomena, and the characteristic condensation of the meganucleus cannot be regarded as an intrinsic part of the process of fission alone. It is of interest to note, furthermore, that the meganucleus in no case takes the initiative; the cytoplasmic change invariably precedes the karyoplasmic, thus reversing the order of events prevailing in cell-division in Metazoan- and plant-cells.

I have made repeated experiments to ascertain whether membranellæ could be regenerated in any except the ordinary way. The adoral zones of Stentors were damaged with the needle in various ways and at different points, and portions of the zone were amputated with the scalpel. If the injury was slight, the wound closed up, bringing the cut ends of the zone into contact, so that the zone was, apparently, as good as ever. If enough of the zone was cut away to interfere seriously with its function, membranellæ were never formed in place of the lost ones; the loss was made good by regeneration in the usual way. The result of the experiments led me to the conclusion that the regeneration of the oral structures of Stentor does not take place *in situ*. As would be expected, injury to or removal of the *adoral* portions of the zone, especially if affecting the pharynx, were more likely to cause regeneration than injuries to the *aboral* portions.

Just as among pluricellular beings the regeneration of highly-specialized organs always takes place from the least differentiated cells available, so among unicellular organisms the more specialized portions ("organula") of the cell are regenerated

from the less specialized. Thus, in *Stentor*, the membranellæ are developed from the indifferent endoplasm, and in a place remote from their final position.

D. CONJUGATION.

My observations on the conjugation of *Stentor* are very fragmentary, for I have been unable to obtain sufficient material for a complete study of it. Conjugation in all species of *Stentor* is apparently a rare event. It was observed by Stein ('67, p. 217) in *S. niger* only; by Moxon ('69) in *S. cæruleus*; and by Balbiani ('61, '82, '92), who has been the only one heretofore to make any study of the nuclear phenomena of the process, in *S. cæruleus* and *S. roeselii*. I have found a few conjugated individuals of *S. cæruleus* and *S. igneus*. In spite of the fact that I have had many large colonies of the Blue *Stentor* for periods varying from two weeks to four months, I have never observed an "epidemic" of conjugation such as Balbiani ('82, p. 161) says he has seen repeatedly in case of *S. cæruleus* and *S. roeselii*.

External Phenomena.—The only conjugates of *S. cæruleus* that I have obtained were found May 12–15, 1891, in a small, ill-fed colony that had been in the laboratory for ten days. Every individual in the colony was examined repeatedly during the four days that conjugation prevailed, but out of two or three hundred *Stentors* only fifteen couples were found.

The *Stentors* in the culture were nearly all medium-sized or small, and, as food was scarce, were very free from food-vacuoles. Usually the gametes were unequal¹ in size—in one instance very strikingly so (Fig. 50, Pl. XXVI.). The mode of attachment I have found to be exactly as it was long ago described by Balbiani ('61), implicating only a small area in the left-hand portions of the frontal fields of the gametes, and thus compelling them to face in opposite directions, as it were. The line of suture appears as a dense, glistening vertical band (Fig. 49), and sections show that it is not merely a superficial line, but a plate covering the whole surface of contact, and

¹ Out of the 15 pairs, the gametes of 9 were notably unequal in size.

evidently formed from the ectoplasm, as it has the dense consistency of that layer and contains abundant pigment. At the time of interchange of the micronuclear spindles, an opening must, of course, be formed in the plate.

The behavior of the gametes shows little that is characteristic. They much prefer to remain fixed in one spot, when they become moderately extended, and stand at a wide angle to each other, the attitude being strongly suggestive of an effort to pull apart. If disturbed, the gametes swim easily, rotating about the long axis in precisely the same manner as the individual Stentor; if there is considerable difference in size, the larger gamete fairly carries the smaller about. The attachment of the gametes is very firm; not only is it maintained during their violent contractions, but they can be sucked up into a pipette without danger of separation.

As in case of *S. cæruleus*, I have only once observed the conjugation of *S. igneus*. In July, 1891, I found a few conjugated pairs at Wood's Holl, at different times from the 17th to the 25th. As usual, the gametes were of small size (in one instance of the variety *nigricans*), and nearly all showed a notable development of red pigment in the anterior part of the body. The manner of union and relative position of gametes does not differ from that of other Stentors, but the area of attachment is proportionally greater than in *S. cæruleus*.

Nuclear Phenomena.—Something of the internal processes of conjugation can be seen in the living specimen. Such observations are greatly facilitated by the absence of food-vacuoles.

One of the most noticeable changes of those observable in the living animal is the separation of the nodes of the meganucleus. This occurs very early in the process of conjugation. It is obviously brought about by rupture of the commissures, which, as in division, are promptly resorbed by the nodes. The latter then become perfectly spherical. Their structure undergoes no change at this time, and their affinity for stains is as strong as ever. After separation the nodes leave their place in the peripheral portion of the endo-

plasm, and, caught in the endoplasmic cyclosis, are carried to all parts of the animal. It is a striking fact that the protoplasmic circulatory movements are far more active at time of conjugation than at any other period in the life of Stentor that I have observed. The arrangement of cytoplasmic threads about the separated nodes of the meganucleus (Fig. 51) reminds one strongly of the streaming reticulum in *Noctiluca* or in the stamen-hairs of *Tradescantia*.

Micronuclei of different sizes and stages of development can be seen in the living gamete, and the movement of these is quite as active as that of the nodes. They have a glistening, refractive appearance. I was never able to see a micronuclear spindle in the living animal, the most of the micronuclei observed being probably already developing to become meganuclei (Fig. 50, *mgn.*¹?).

By reason of the difficulty in obtaining material, and not less, perhaps, on account of the obscuration of the micronuclei by the comparatively great mass of cytoplasm, no adequate account of the complex details of conjugation in any species of Stentor has been given. The latter difficulty I have obviated by sectioning the gametes, which can be readily oriented. I have endeavored to secure material by the method used by Maupas ('89, p. 168), but without the least success.¹

While it would be impossible to interpret the results I have obtained from the small amount of material at my command, if we had a less complete knowledge of conjugation in other forms, I believe it may be done in the light of the beautiful researches of Maupas ('89) and of R. Hertwig ('89) in this field.

Nearly all of my preparations show that the gametes were in the later stages of conjugation (Stage H of Maupas), and consequently I have not seen the exchange of micronuclear spindles. In only one preparation have I found spindles, two of different shape (from the same gamete) being represented in Figs. 53, 54. They are both in the "monaster" or "mother-star" stage, and have longitudinal, linear chromosomes which

¹ I have also tried Maupas' method with *Paramecium caudatum*, which multiplied enormously in hay infusion, and was afterwards subjected to long periods of fasting, but obtained no "epidemics" of conjugation.

coincide with the very distinct spindle-fibres. The spindle is enormously large (length, 16μ) for the size of the resting micronucleus (Fig. 55). It is considerably larger than the spindle formed at time of fission.

In conjugates at a late stage and in ex-conjugates, spherical, translucent, unstainable bodies are found, varying in size from 8μ to 12μ . (Figs. 57, 58.) These are undoubtedly the anlagen of new meganuclei. Their behavior towards staining reagents is very characteristic, for I have not noticed the least tendency to take a stain with picro-carmin or Czokor's alum cochineal. This is in perfect accordance with the observations of Gruber ('87), Maupas ('89), and R. Hertwig ('89). The unstainability of the meganucleus during its evolution is due either to an absence of chromatin, or to a chemical change in it; the latter seems the more probable, for both in the micronuclear state and in the condition of a fully-developed meganucleus these bodies contain abundant, highly-stainable chromatin.

The general appearance presented by the nuclear structures of two gametes in Stage H is shown in Fig. 52. The separated nodes of the old meganucleus have not as yet undergone (in this particular example) any perceptible alteration of structure or loss of affinity for stains. Numerous micronuclei are present, distributed throughout the cytoplasm. They are much enlarged beyond the minute dimensions they have in the resting state (compare Figs. 55 and 56), and stain less deeply. They have a somewhat granular structure, with occasionally a suggestion of chromatic threads (Fig. 56). Besides micronuclei, the anlagen of new meganuclei are found (*mgn.*¹)—in this instance as in some others, of very different size in the two gametes. Although, according to Balbiani's observations ('61, '92) only a single meganucleus develops in each gamete, I have observed that several often pass through the early stages of the evolution. We must, therefore, assume that the superfluous ones are absorbed by the cytoplasm, and so only one reaches the final stages of development.

Still other structures found at this stage are micronuclei within vesicles two or three times their diameter. (Fig. 52, lower gamete, Fig. 59.) These I agree with Maupas in

regarding as superfluous micronuclei undergoing digestion. The vesicle may be compared to a digestive vacuole.

In the later stages of conjugation, the separated nodes of the old meganucleus stain less deeply, and begin to lose their spherical contour. The example represented in Fig. 60 is from an ex-conjugate, being from the same specimen as Fig. 57. The chromatic reticulum is still existent. At two points clear vesicles, probably of karyoplasm, cause the nuclear membrane to bulge out. I have never seen such vesicles in the ordinary state of the meganucleus of Stentor.

E. TERATOLOGY.

As Balbiani has remarked in a recent paper ('91), the teratology of Protozoa is an almost untouched field of investigation, and is certainly a promising one. Balbiani's own contribution ('91) to the subject is based upon the study of two double monsters of *S. cæruleus*, one of them artificially produced by merotomy. In a still more recent and very suggestive paper ('92), the eminent French micrographer has shown how double monsters of Stentor may be produced at will by the merotomy of specimens in the earlier stages of fission. My own results are also derived from the study of a double monster of *S. cæruleus*, one which was not produced artificially.

When discovered, the specimen presented nearly the appearance shown in Fig. 61A, with the exception that the anlagen of new zones (*a.z.*,² *a.z.*3) had not appeared. The animal has the aspect of being in a stage of fission soon after the appearance of the constriction. The new adoral zone (*a.z.*1) had, however, no mouth at its lower extremity, although the place for it was marked by a deep fold, into which the posterior extremity of the new zone penetrated. Another peculiarity is the reversed position of the anterior zoöid. The twist is plainly indicated in the direction taken by the stripes, which apparently present a distinct system for each zoöid, meeting each other at a wide angle in the fission-line. As the ramifying zone¹ of the anterior zoöid is placed far to the right of its normal position, it is evident that the torsion has been from left to

¹ Its position is indicated by the new adoral zone *a.z.*3.

right. The meganucleus was already in the noded condition, and as nearly as could be made out in the living animal, was in two pieces, one of ten nodes lying in the proximal zoöid, the other of twelve nodes lying for the most part in the distal. A contractile vacuole was present in each zoöid.

The first change observed was the appearance of two new zones (Fig. 61A, *a.z.*², *a.z.*³), one for each zoöid. That on the posterior zoöid (*a.z.*²) appeared a little before the other, but the evolution of the two went on very nearly *pari passu*. Three hours later a new mouth had appeared at the lower extremity of each, (*o.*², *o.*³) and at the same time the meganucleus had undergone condensation into three or four masses. Six hours after the appearance of the new zones, *o.*² had been drawn up into the position of the absent mouth of the posterior zoöid (Fig. 61C), and at nearly the same time *o.*³ had taken the place of *o.* Another change which had probably been in operation since the finding of the specimen, had by this time become very evident: viz., the forward movement of the whole posterior frontal field and zone to the plane of the anterior. Possibly it would be more accurate to describe the change as a *withdrawal* of the anterior zoöid into the posterior. Whichever movement took place, its important result was to bring the two frontal fields to the same level, and therefore the two parts of the double monster into closer union.

The next observations were made 15 hours later, and revealed a great change in the appearance of the monster (Fig. 61D). It now had one immensely large frontal field composed of the two preëxisting frontal fields, and encircled by an adoral zone made up of repeated neoformations arising at two different places on the body. The patchwork character of the zone is clearly marked by the numerous breaks in the series of membranellæ. The dualism of the specimen is still manifest in the arrangement of stripes in the frontal field in two distinct systems, in the development of two more new zones, and in the two contractile vacuoles (*c.v.*, *c.v.*¹).

A point of much interest is the *contemporaneity* of the oral renovation, now observed for the second time in this specimen. In both instances the synchronism in the neoformation of oral

organs is the more striking, inasmuch as there seems to be no occasion for the regeneration of more than one of the zones. At the stage represented by Figs. 61A, B, it is the mouth on the left (*i.e.* posterior) that is wanting, while in the later stage (Fig. 61D) the mouth on the right is absent. The only explanation of this synchronism in the development of duplicate parts seems to be a close coördination of functions throughout the dual organism, which therefore *physiologically* is to be regarded as a single individual, but with duplicate organs and functions.

The double Stentor underwent little modification during the next nine hours, in the course of which it was frequently examined. The new mouths (*o.*⁴, *o.*⁵) were gradually drawn into position, and *o.*² atrophied. By the following morning, 25 hours after Fig. 61 D was drawn, a great change had taken place (Fig. 61 E), amounting to a loss of dualism, and therefore a return almost to the normal condition. The right-hand mouth (*o.*⁴) had atrophied. The breaks in the zone had, with one exception, disappeared; the frontal field was much reduced in size, and its two systems of stripes had nearly melted into one. Only one contractile vacuole (*c.v.*¹) was present. The regeneration (*a.s.*⁶) of the oral apparatus (*o.*⁴) was in progress, but no similar regeneration was under way for the atrophied *o.*⁵—strong evidence of the loss of physiological duality.

The subsequent modifications of our Stentor were unimportant, and consisted mainly in the obliteration of every trace of duality. After a day or two it differed from normal Stentors only in the slightly greater size and oval shape of the frontal field. No fission occurred during the four or five days I had the Stentor under observation.

One of the double monsters studied in detail by Balbiani ('91) differed from mine in that duality was present, not in the anterior, but in the posterior portion of the body. The monster was furthermore artificially produced by amputating the frontal field and dividing the posterior part of the body by a longitudinal incision, so as to give it a bipedal appearance. The regeneration of the frontal field was not observed. The interesting point of likeness between Balbiani's specimen and mine is the gradual obliteration of duality and return to the

normal' form. A striking fact in the history of Balbiani's monster is its fission *previous* to the disappearance of its bifid structure, which it therefore transmits to its proximal offspring. There was, however, not the slightest development of duality in the distal offspring; even in the proximal it quickly disappeared, and the normal form was regained.

In the light of Balbiani's most recent experiments upon Stentor ('92), I am led to believe that double monsters of this species, and doubtless of Infusoria in general, owe their origin to the temporary dualism of the cytosome at time of fission. If through imperfect development or mutilation the normal progress of fission is interfered with, the dualism already set up in the cytoplasm does not fail to manifest itself in the production of a double monster. Some of Balbiani's figures (especially Fig. 7b¹-b 5, Pl. II, '92), show the gradations between a nearly-divided merozoan (originally cut from a Stentor in fission), and a perfect double monster with two frontal fields.

The duplication of newly-formed organs in both zooids in fission is another interesting instance of dualism, comparable to the simultaneous appearance of new zones in a double monster. It was observed by Stein ('67) and carefully studied by Sterki ('78) in the neoformation of marginal cilia and of cirri in both daughter-individuals of *Stylonichia*.¹

The position taken by the meganuclei in a double monster is worthy of notice. A nucleus is usually present in each moiety

¹ The regeneration of ciliary organs in the Oxytrichina is of especial interest. These organs, as is well-known, are highly differentiated, occurring in form of cirri, spines, etc., which are definite in number. In regard to their development, the work of Stein and of Sterki has shown that the frontal, ventral, and anal cirri by no means originate in their definitive form and position. Their earliest appearance as a group of six parallel rows of minute, similar processes, from which the cirri develop and are shifted to their final positions, is very suggestive of the primitive six rows of cilia of the lower Hypotricha (*e. g.*, *Urostyla*). The point of chief interest in this connection is, that new cirri are formed, not alone for the posterior zooid, but also for the anterior, where the old frontal and ventral cirri atrophy and are replaced by the new ones. In the posterior zooid, on the other hand, the caudal cirri of the parent are replaced by the newly-formed cirri. Just as in Stentor, the neoformation of a structure demands the atrophy of its preëxisting homologue.

of the body; or if there is but one, it extends into both (Fig. 61 A).

IV. BIOLOGY AND PHYSIOLOGY.

I have set apart this portion of the present paper for an account of certain experimental work and physiological observations. Many of the results are obviously far from complete; but are, I believe, not without interest, and will at least serve to show how well-adapted are the Stentors for various lines of biological and physiological experimentation.

A. *Rate of Multiplication.*

Most species of Stentor do not multiply readily in confinement. This is especially true of the chlorophyll-bearing forms, none of which have I seen in fission more than a very few times. But, considering the enormous colonies these species form in favorable localities, multiplication must be more rapid under natural conditions. The most prolific species that have come under my observation are *S. cæruleus* and *S. roeselii*. If food be abundant, a few individuals of *S. cæruleus* will in a week or so become the progenitors of thousands and fairly overstock the aquarium.

After reading Maupas' account of his highly-successful experiments upon the multiplication of Infusoria ('88) I determined to test the results he obtained from the propagation of the Blue Stentor (p. 229). I followed his method closely, using the excellent moist chamber that he recommends (p. 179), and making the same sort of slide-cultures. For these I used cover-glasses 18 mm. square, and elevated about a millimeter from the slide with wax feet. For food I raised immense numbers of *Glaucoma scintillans* in hay-infusion. These were fed to the Stentors at intervals of a day or two, and were devoured by them with the greatest avidity. The body of each Stentor soon became gorged with the Glaucomas. Under such generous feeding multiplication was rapid for a few days, often one bipartition to every 24 hours, at a temperature varying from 12° to 22° C. But that this rapid increase was

not, as Maupas supposes, a normal rate, was shown by the fact that it could never be maintained for any length of time.

I soon became convinced that the slide-culture method, however successful it might be with other species of Infusoria, was not adapted to Stentor. So I made cultures in large watch-glasses, each covered with another watch-glass to prevent evaporation. Most of the watch-glass cultures were supplied, in addition to the food-infusoria, with unicellular green algae, usually a species of *Scenedesmus*. The rapid growth of these algae kept the water pure, and to some extent furnished food for the Stentors. But the watch-glass cultures were only in a slight degree more successful than the slide-cultures; for while I was able to raise Stentors on the slide to the 7th or 8th generation, I did not succeed in getting any colony in a watch-glass beyond the 10th.

One of the most rapid multiplications that I have noted was of a culture started in a 2-inch watch-glass with a single Blue Stentor, Dec. 1, 1891. Following is the record of the culture:—

		INDIVIDUALS.				BIPARTITIONS.			
Dec.	2	.	.	.	2	.	.	.	1
(One was isolated.)									
"	4	.	.	.	2	.	.	.	2
"	5	.	.	.	4	.	.	.	3
"	7	.	.	.	8	.	.	.	4
"	8	.	.	.	16	.	.	.	5
"	9	.	.	.	32	.	.	.	6
"	10	.	.	.	58	.	.	.	7

Temp. 15°–22° C.

It is seen that there were two intervals when bipartitions were 48 hours apart, whereas the rest were at the minimum interval of 24 hours. After attaining the 7th generation the Stentors almost ceased to multiply and many died. The culture having received abundant feed from beginning to end, lack of nutriment could not be considered as the cause of its decadence.

What was the effect of such frequent bipartitions upon the individual? Neither in this nor in any other instance of

equally frequent bipartitions that I have observed accurately, has the size of the original progenitor been maintained. After only two or three consecutive fissions at 24-hours' interval, the reduced dimensions of the offspring become very noticeable. I believe the cause to be a lack of sufficient time between the bipartitions for the offspring to attain the size of the parent, and consequently after the next bipartition the grand-children, so to speak, are only a little over a fourth the size of the grand-parent, whereas they should normally be half its size. As the culture described above had undergone the most rapid multiplication, its members were reduced to the most minute dimensions. They became mere dwarfs, measuring at the 7th bipartition only .630 mm. (extended) by .285 mm.,¹ and were rapidly passing into a lethargic condition.

On Dec. 16 they were again examined. I had repeated the observation made by Maupas—that inability to take food did not altogether prevent fission; but such fission was of course not followed by any growth of the offspring, and therefore produced a rapid reduction in size. The few Stentors still living were in a moribund state. The movement of the membranellæ was hardly perceptible. In most instances the mouth parts were nearly or wholly atrophied. The average size was .450 mm. in length, and .120 mm. across the frontal field. On staining specimens with methyl-green, I found the nodes of the meganucleus only 5–10 in number, and much reduced in size, although still very large in proportion to the size of the animal, which consequently possessed an abnormally large amount of nuclear substance in proportion to the cytoplasm. The nuclein took as deep a stain as usual.

My most successful culture was made in a large watch-glass kept supplied with fresh water to make good that lost by evaporation, not over-supplied with food (*Glaucoma scintillans*) and furnished with minute green algae, which grew freely and kept the water well oxygenated. The temperature was variable, falling to 12°–15° C. at night, and rising to 22° during the day. The record is as follows :—

¹ The normal size of the species, it will be remembered, is 2 mm. by .5 mm.

ONE BLUE STENTOR, ISOLATED DEC. 3, 1891.

	INDIVIDUALS.								BIPARTITIONS.
Dec. 4	.	.	.	.	2	.	.	.	1
" 6	.	.	.	.	4	.	.	.	2
" 8	.	.	.	.	8	.	.	.	3
" 9	.	.	.	.	7 (one died)	.	.	.	3
" 10	.	.	.	.	14	.	.	.	4
" 11-15	(Increased rapidly; not counted.)								
" 16	.	.	.	.	220	.	.	.	8
	(One was isolated.)								
" 17	.	.	.	.	2	.	.	.	9
" 19	.	.	.	.	3	.	.	.	9½
" 22	.	.	.	.	4	.	.	.	10
" 23	.	.	.	.	4	.	.	.	10

My unavoidable departure from Worcester at this time brought the culture to an untimely end. It will be seen that the usual rate of increase was one bipartition in 48 hours; that all the individuals of a given generation (especially during the earlier bipartitions) divide at about the same time; that the rate of increase is accelerated from the 4th to the 9th bipartition; that, finally, discrepancies in the times of fission of the offspring of each bipartition appear, and increase at each subsequent fission. In passing from the 9th to the 10th bipartitions the interval between the fission of the two individuals amounts to nearly four days.

My culture experiments, though unsuccessful in producing a long series of generations like the well-known experiments of Maupas, at least demonstrate that it is possible to stimulate Infusoria to a rate of increase beyond the normal, leading to speedy deterioration of the race. It is probable that Stentors are somewhat exceptional in this respect, and that such species as *Stylonichia pustulata* and *Onychodromus grandis*, in which the time required for fission is much less than in Stentor, are less susceptible to the pathologic effects of over-fecundity; but these effects should, nevertheless, be taken into account.

Since under rapid multiplication a Stentor culture deteriorates, under what conditions does the individual Stentor attain its best development? I gained a hint in the right direction upon examining an old culture that had stood

undisturbed for weeks. I had originally placed in it a large number of Blue Stentors from an unusually rich gathering collected in Alewife Brook, Cambridge. The culture had not done well, apparently from lack of food, and I believed that it had become barren. Examination showed that it contained a few Stentors of unusually large size and splendid development. The cytoplasm was very free from food-vacuoles, and it was evident that they had had a meagre diet for a considerable time. This I believe to be the prime cause of their large size and fine development—a regimen sufficient to maintain life and admit of growth, but not generous enough to promote fission. Several of the Stentors were placed in a watch-glass which already contained an abundant growth of *Scenedesmus*, upon which they fed. Fission took place only at long intervals, and the large size of the progenitors was maintained in their descendants.

It will perhaps be claimed that the large size and fine condition of the Stentors above-described was due, not to meagre diet, but to the fact that they were the survivors of a much larger number, and according to the doctrine of natural selection, must have been the most vigorous and best able to cope with adverse conditions. There is certainly something to be said in favor of this view. But an experiment hereafter to be described (see p. 540) has shown that the largest Stentors are not necessarily the most vigorous or prolific, and another experiment has demonstrated that it is possible to take Stentors of average size or less, put them on a scanty vegetable diet, and obtain large and finely-developed specimens. The details of the latter experiment are as follows: On Jan. 15, 1892, I found a Stentor (of about average size) in fission, which showed the division of the meganucleus at time of condensation. I placed its offspring in water which contained a growth of minute algae. On the 16th another Stentor, also of average size, was found, and it likewise exhibited the early division of the nucleus. Its offspring were placed with the others, the object being to ascertain whether the early division of the nucleus would reappear at subsequent fissions. The original four Stentors had increased to six on the 18th and to eight on

the 20th, but no further fission took place. The Stentors fed upon the algae, but obtained very little else. On Feb. 3 the culture was examined, and the Stentors were found to be much increased in size and finely developed. In the interval of two weeks during which there had been no increase in number, a slow growth had taken place.

B. *Relative Vitality of Large and Small Stentors.*

It is a fact familiar to all who have observed Infusoria that there are many species individuals of which vary much in size. These differences are due to various causes, such as rapid multiplication without intermediate growth-periods, multiple fission within the cyst, bud-formation, scarcity of food, etc. Hardly any forms show such an astonishing variation in size as the Stentors, and as we have seen (p. 537), this is especially true of *S. cæruleus*.

It seemed to me probable that the dwarf Stentors were not necessarily enfeebled and dying members of the colony, and I surmised that under favorable conditions (which they certainly do not have in an aquarium inhabited by multitudes of their larger kindred, which overreach them and obtain most of the food), they would increase in size and prove as prolific as the larger ones. In order to test the truth of my supposition, I arranged the following simple experiment. On the 21st of March, 1891, I started eight colonies in small beakers, each containing 20 cc. of water, taken from a gathering made less than a week previously. Great care was taken not to introduce any Stentors with the water. Into each of four beakers ten of the largest Stentors obtainable were put, and into each of the remaining four, ten of the smallest. No Stentors in process of fission were introduced. The beakers were placed upon a table out of direct sunlight, and those containing Stentors of different sizes were set abreast, two and two. The relative positions of the pairs were changed at intervals, those towards the light being placed away from it, and *vice versa*. Thus nearly all the conditions—light, temperature, size and shape of dish, quantity, quality, and depth of water—were made as uniform as possible. But one most important

factor, that of food, was not uniform, and it seemed impossible to make it so. While at the outset all the cultures probably contained nearly the same amount of available nutriment, the more extensive growth of plant-life and the greater multiplication of minute organisms (*e.g.* Arcella) in some of the cultures, placed the inhabitants of those cultures at great advantage. Hence the wide difference in the number of Stentors in the different cultures.

A month later a census was taken of the colonies, with the following result :—

	NO. OF STENTORS.	REMARKS.
No. 1 (large),	30	Large to small ; well-fed.
2 (small),	31	Mostly small ; not well-fed.
3 (large),	5	Small ; moribund.
4 (small),	55	Large to small ; well-fed.
5 (large),	40	Average size ; well-fed.
6 (small),	5	Average.
7 (large),	43	Large to small ; mostly well-fed.
8 (small),	144	Large to very small ; well-fed.

The record shows an increase for all colonies except Nos. 3 and 6, which obviously were suffering from lack of food. The great variation seen in the increase of the other colonies is doubtless almost entirely due to the great difference in the amount of available food. But the experiment at least shows that the dwarf Stentors are not incapable of growth, and under favorable circumstances will multiply as rapidly as large ones ; for, comparing the cultures by twos (No. 1 with No. 2, No. 5 with No. 4, No. 7 with No. 8), we find in every instance a balance in favor of the small Stentors.

It is worthy of note that although the ten brood Stentors used in starting each culture were very nearly alike in size, their descendants soon came to show the usual marked difference in that respect.

Very interesting in this connection are the recently-published observations of Gruber ('92) upon dwarf Stentors. The occurrence of a natural colony of Blue Stentor dwarfs, all of which, as far as examined, possessed a simple nucleus the size and shape of a single node of the usual moniliform nucleus, was

certainly a remarkable fact, and strongly suggests the possibility that the supposed "dwarfs" were in reality a distinct species, especially since no transitions were found from the dwarf to the Stentor of normal size with moniliform nucleus.

C. *Nutrition ; Alimentary Vortex.*

I have made a few observations upon the beautiful method of capturing food prevailing among the Stentors—a method of wide occurrence among the Infusoria, which Maupas has fittingly termed the *alimentary vortex* ("tourbillon alimentaire"). The appearance of the alimentary vortex is shown in Fig. 62. The carmine-grains supplied in liberal quantity were eaten freely, as the drawing clearly shows. The little arrows indicate the direction of the currents created by the vibration of the membranellæ. It is impossible to detect the direction of the "strong beat" of the membranellæ by an inspection of these organs in action, but it is revealed indirectly by the course of the currents, which in turn are made manifest by the movement of the carmine-grains. Since, then, the particles move in an ascending curve to the left from positions of rest to the right of the zone (when viewed, of course, from the dorsal side, as in Fig. 62), and then sweep directly downward upon the frontal field, we must infer there is some sort of suction towards the frontal field, which could only be brought about by a strong *inward* stroke of the membranellæ. But this is combined with another stroke, the direction of which is *along* the zone towards the mouth. The result is that all particles coming within the sphere of influence of the alimentary vortex are drawn down to the frontal field, over the surface of which (probably propelled by the motion of the minute cilia), they glide towards the oral orifice. In *S. roeselii* I have seen the slow movement of nutrient particles along the marginal channel characteristic of that species ; but in *S. cæruleus*, although the elevated adoral zone (Fig. 62) effectually prevents the food-particles from slipping over the edge of the frontal field, I have not noticed that the carmine-grains took any particular path on their way to the mouth.

Having reached the extreme left-hand portion of the frontal field the grains drop down into the buccal pouch (*b.p.*), within which they are rushed back and forth several times, until finally they slip into the inner turn of the spiral, thence through the mouth opening at its apex into the endoplasm (see arrows in Fig. 62). I have once or twice seen the grains take a definite path through the endoplasm, so as to suggest an œsophagus, but usually one sees nothing to indicate the presence of one. I have not seen the "Schlundstrang" described by Schuberg ('90), either in the living animal or in sections. Schuberg himself says it is in evidence only at the time fine food-particles are being taken in.

The grains of carmine speedily become distributed, either as single grains or little clusters, throughout the anterior part of the animal, but penetrate more slowly into the posterior portion. The dissemination is evidently brought about by the circulatory movements of the cytoplasm. On examining a specimen that had recently been fed with carmine, I was unable to detect that the carmine-masses lay within digestive vacuoles, and repeated scrutiny failed to reveal any vacuolar space around the food-masses. I do not think, however, that a digestive vacuole was absent, but believe it was either very small or wholly obscured by the dense cytoplasm.

The manner of capturing a living prey is different from the almost mechanical inception of non-motile food-particles. I have observed the capture of a small "skipping" *Hypotrich* of undetermined species. Notwithstanding the rapid and vigorous swimming powers of these little Infusoria, they are frequently entrapped in the powerful vortex of a Stentor, and thereby get drawn into the buccal pouch. Instantly the lips of the pouch (velum and buccal fold) close nearly together, making escape almost impossible. The captive then darts from end to end of the pouch. After a few seconds of this shuttle-like motion, the prey is driven into the inner spiral, thence through the oral opening, always accompanied by a bulk of water several times its size, into the endoplasm. The vacuole thus formed is speedily carried away from the oral aperture, but the Infusorian within still darts about for a minute or so before

death ensues. Almost immediately after death disintegration sets in.

It would be hardly possible for any Infusorian to have a more extensive bill-of-fare than the Blue Stentor. It devours not only Protozoa and Protophytes of almost every group, but small Rotifers as well. The latter, together with Paramecium, *Stentor igneus*, and dwarfs of its own species, are among the larger prey captured by this voracious animalcule. The booty sometimes equals nearly one-third the bulk of its captor. I have never observed the deglutition of these huge morsels; they must dilate the very distensible oral spiral to the utmost. The cannibalistic habit of *S. cæruleus* is not without interest. It shows how large and vigorous members of a colony may survive a period of scarcity, and at the same time reduce the number (always large at such times) of the dwarfs in the community.

D. Defecation; Position of Anus.

One of the favorite doctrines of micrographers is that of a localized place of defecation in the Infusoria. It is not commonly pretended that this so-called "anus" is a permanent structure, or visible at any time except at the evacuation of fœcal matter. An anus, then, among Infusoria is held to be not structurally but physiologically present.

But are we certain that there is a fixed place of defecation? Observations on this point are rather difficult to obtain, and often require long and patient watching. Consequently, the number of observations for any one form are generally very few; and, to make the matter still more doubtful, observers are by no means unanimous in their statements regarding the position of the anus in one and the same species. This is commonly held to be due to careless or incorrect observation. May it not rather be due to variation in the place of defecation?

My study of the defecatory process in the Stentors has led me to think that the temporary anus of the Infusoria is not so fixed in position as is commonly held. I have many times observed the evacuation of fœcal matter by *S. cæruleus* and *S. roeselii*, and have, as a rule, found that it took place in the

region indicated by Claparède and Lachmann ('59), Stein ('67), and Moxon ('69)—on the dorsal side at the same level with, and a little to the right of, the contractile vacuole. (Fig. 63, *a*.) But the evacuation of excreta is by no means always at this place; sometimes they are extruded at a spot but slightly removed; sometimes at places far distant, as at the lower part of the dorsal region, or on the right and left sides of the animal (Fig. 63, *fc*). These observations have been made upon animals not under cover-glass pressure, but living under free conditions. It cannot, therefore, be supposed, as Bütschli ('89, p. 1386) has done in case of a similar observation by Brauer upon *Bursaria truncatella*, that the evacuation of excreta in places other than the usual is due to pressure. But a statement should be made regarding the character of the substance discharged. It consisted in most instances of minute green algæ (in the example shown in Fig. 63, of *Scenedesmus*), which *S. cæruleus* sometimes devours in large quantities, but to all appearances does not digest; for the algæ are discharged in as bright and green a condition as they are taken in. Hence the evacuation of such ingesta is not the same thing as the discharge of the residue of digestion. But if undigested excreta can be extruded through the pellicula at various points, can we venture to assert that faecal matter may not also be eliminated at more than one place?

Since in most Infusoria¹ no morphological structure indicates the place of the anus, why is defecation for the most part confined to a particular region of the body? It is unquestionably due to the fact that, after digestion is completed, faecal matter is brought by the slow streaming movement of the endoplasm to one particular place, and there accumulates until the time of defecation. In *S. cæruleus* a large excrement-vacuole may be seen lying near the dorsal surface of the body, about at the level of the contractile vesicle. I have seen two excrement-vacuoles in this region come into contact and then

¹ Maupas ('83, p. 650) has expressed the view that the anus is permanently present as a cleft in the pellicula, which opens only at time of defecation. Bütschli ('89, p. 1387) adopts this view, and even finds in *Balantidium* a permanent anal pore. An anal tube was figured and described by Stein ('67) in *Nyctotherus*.

flow together into one ; and it seems highly probable that this is the mode by which fæcal matter distributed in vacuoles throughout the body is gradually collected into one large excrement-vacuole.

As to the process of defecation, Moxon ('69) says : "Not far from the opening in the contractile vesicle in *Stentor* is the point of surface which gives exit to the solid residues of the creature's food. . . . Its place is marked, not by any pore or opening, but by an irregularity or break in the course of one or two of the longitudinal blue bands before described. . . . The mass to be thrown out is brought to the surface and simply forced through it, sometimes carrying with it a thread of the glairy contents of the saccular body of the animal." I have observed no interruption in the course of the stripes at the usual place of defecation, and cannot regard it as a characteristic feature. The "break" in the stripes is rather to be looked upon as the cicatrix resulting from a previous rupture of the integument at that point.

Concerning the extrusion of fæcal matter, two things are to be noticed : (1) the contractile power of the cytoplasm surrounding the excreta, (2) the rupture and subsequent closure of the pellicula and ectoplasm. The contraction of the endoplasm around the excreta is comparable to the contraction of that about the contents of the pulsating vacuole, but its action is certainly slower and less vigorous. Yet it is sufficiently perfect to eliminate every particle of excreta in the vesicle, but usually, as Moxon states, with the loss of some of the cytoplasm.

Inasmuch as defecation can occur with equal success at many points it is evident that the contractile power of the cytoplasm is diffused, not restricted to one place, as we should probably think to be the case if we considered the contractile vacuole alone.

The rupture of the integument is an interesting process. There first appears over the excrement-vacuole a longitudinal slit through pellicle and ectoplasm, exposing the excreta. The lips of the slit rapidly spread apart, and in a moment lay bare the whole exterior surface of the mass of excreta, even though

it be half the breadth of the animal. While the integument has been opening, the contractile power of the endoplasm has been gently forcing the mass outward. (Fig. 63, *fc.*) The process often requires several minutes. As soon as the entire mass has passed beyond the periphery of the animal's body, the lips of the opening approach slowly, and completely cover the exposed place.¹

E. *Contractile Vacuole.*

I have made several observations upon the rate of pulsation of the contractile vacuole in *S. cæruleus* and *S. roeselii*, and find an unexpected amount of variation therein. This variation cannot be accounted for by difference in temperature, for it appears in consecutive pulsations of the same vacuole, when no sensible change in temperature has intervened.

The mode of discharge of the contents of the contractile vacuole of *S. cæruleus* was clearly and accurately stated by Moxon ('69); and the method in which the fluid is collected, and the changes in form of the vacuole during diastole have been elaborately described by Maupas ('83, p. 641).

In regard to the intervals between the systoles of the contractile vacuole, they have seemed to me to show variation characteristic of individuals rather than a variation due to change of temperature. The consecutive intervals, besides, are very unequal. Thus, one individual of *S. roeselii* observed by me had a vacuole which contracted five times at intervals of 50 seconds, the air-temperature being 20° C. Another, at 17° C. gave a record of four consecutive pulsations at intervals of 1 min. 20 sec. Still another contractile vesicle pulsated four times at unequal intervals: 1 min. 15 sec., 2 min., 1 min. 40 sec., 2 min. 10 sec.,—showing thereby a difference of 55 seconds between the first diastole and the last. Unfortunately,

¹ Recent observations have led me to think that the mode of defecation is influenced considerably by the nature of the excreta. When it is coarse and matted together, the integument must open widely, as above described. But when it is composed of minute, smooth, rounded bodies, a small opening is formed, and the excreta forced through it in a stream.

the temperature was not noted. Another, at temperature of 22° C. gave the following remarkable record :

1 min. 47 sec.	1 min. 43 sec.
1 " 43 "	1 " 47 "
1 " 43 "	1 " 43 "
2 " 0 "	2 " 40 "
1 " 50 "	

The irregularities in pulsation are quite as marked in *S. cæruleus* as in *S. roeselii*. At 19° C. I have noted an interval of 2 min. 20 sec. for four consecutive contractions. Another specimen yielded the following record (temp. 21° C.): 3 min. 30 sec., 3 min. 27 sec., 3 min. 35 sec., 3 min. 30 sec., 3 min. 25 sec., 3 min. 30 sec. We here detect what has been apparent in some of the foregoing records—a tendency to alternation of long and short diastoles, and constant recurrence of periods of same duration. It will be also noted that the rate of pulsation is much slower in *S. cæruleus* than in *S. roeselii*, whatever the temperature.

It is apparent that the differences in the rate of pulsation, however dependent upon temperature, are also matter of individual variation, just as we have seen to be the case with fission and regeneration. Hence observations undertaken to ascertain the variation in pulsation of the contractile vacuole coincident with changes in temperature must be made upon one and the same individual, and, furthermore, one in which the pulsations have been found to be isochronous.

F. *Merotomy*.¹

The Stentors have been the subject of extensive merotomical experiments, having been used for this purpose by Gruber ('86), Balbiani ('88), Verworn ('89), and again very recently by Balbiani ('92). My own experiments in merotomy were made upon the Blue Stentor, which is, indeed, the most favorable

¹ The term *merotomy* ("mérotomie") was coined by Balbiani ('88) to designate the operation of artificial division upon unicellular organisms, with the purpose of observing the subsequent fate of the artificially-produced individuals, or "mérozoïtes." In place of this almost unpronounceable word, I propose the term *merozoön*, formed on the analogy of *Protozoön*, etc.

form for this work. While my experiments have been much less extensive and thorough-going than those of Gruber and Balbiani, the results have confirmed theirs with reference to the leading problem: viz., whether the presence of a portion of the nucleus is essential for the regeneration of lost parts and a continuance of the vital functions of a merozoön. Like my predecessors, I have uniformly found that enucleate fragments, even when of considerable size, soon perished without regenerating any of the lost parts. Merozoa containing a portion of the nucleus, on the contrary, were able to regenerate the adoral zone, mouth, and contractile vacuole (Figs. 64 A and 64 B). Such merozoa invariably lost their blue pigment after a day or two, and assumed a tawny color, such as often appears in specimens of this species that are kept under unfavorable conditions. I have never known the blue color to be regained after it is once lost.

Regeneration of the adoral zone takes place in a merozoön in the same manner as in the normal Stentor, and is accompanied by the same meganuclear changes (Fig. 64 A and B). Balbiani's figures ('92) show that an increase in the number of nodes retained by the merozoön usually takes place at regeneration of the adoral zone, just as in ordinary regeneration. But I cannot agree with Balbiani's statement (p. 400) that the increase in the number of nodes is accomplished by the constriction of pre-existing nodes, — a method which he evidently believes to obtain in the normal condition of the Stentor; for he says (p. 400): "*Chez les individus normaux on n'observe point une multiplication aussi active des articles du noyau, dont le nombre augmente seulement de loin en loin par la division d'un quelconque des articles préexistants. Il faut en conclure que, pendant la régénération des mérozoïtes, le fragment de noyau qu'ils contiennent est le siège d'une excitation physiologique particulièrement active, excitation qui se manifeste par les régénérations organiques et la multiplication rapide de ses propres éléments.*"

I have already expressed the opinion (p. 517) that the nodes are increased only at time of renodulation; and the "physiological excitement" of which Balbiani speaks is nothing more

nor less than the ordinary activity incident to regeneration, on whatever occasion it occurs.

As previously stated (p. 526), the regeneration of membranellæ, so far as observed, takes place in only one way—by formation of a new adoral zone on the ventral surface of the animal, normally along the left border of the ramifying zone. Although no definite experiments have been made to ascertain whether nucleate merozoa with no portion of the ramifying zone are able to regenerate the membranellæ, certain figures¹ and statements of Gruber ('86) and Balbiani ('92) make it very probable that such is the case. In his recent work upon the merotomy of Stentor, Balbiani ('92) implies that a new zone, or at any rate a rudiment of one, is sometimes developed *in situ* at the anterior end of the merozoön. It appears to me that in some of these cases the early stages of zone-formation were overlooked, while in others the structures taken for membranellæ are large cilia. I have sometimes noticed a crest of long cilia at the anterior extremity of a merozoön, long before the regeneration of membranellæ appeared.

An experiment, unfortunately a single one of the kind, gave the following interesting result : A Stentor was cut transversely at a point considerably nearer the posterior than the anterior end. A few hours later, the anlage of a new zone appeared on the anterior merozoön. No trace of it had been visible at time of bisection. Believing it to be simply a case of regeneration of the mouth, I paid little attention to it ; but, upon examining the animal two or three hours later I was astonished to find that the new zone was the forerunner of fission. The new mouth was formed in the same place as if the animal were entire, and, therefore, much nearer the posterior end of the merozoön than the anterior. Fission was carried through successfully, giving birth to offspring of very unequal size. An unequal bipartition in *S. cæruleus* is of very rare occurrence ; aside from the above, I have noted but one instance where the daughter-stentors were visibly unequal. The unequal division above noted may then be safely referred to the amputation of the posterior portion of the Stentor. The experiment seems

¹ *E.g.*, Gruber's Fig. 3, merozoa A, C, and Balbiani's Fig. 3, merozoön *b*.

to indicate that the plane of fission is determined before the slightest sign of bipartition is visible, and that the plane is not altered, nor division prevented by the removal of part of the cytosome.

In closing, I would express my sincere thanks to my former instructor, Prof. E. L. Mark, of Harvard, for many favors and valuable suggestions ; and to my present instructor, Prof. C. O. Whitman, through whose courtesy it was my privilege to occupy, during the summer of 1891, an investigator's room in the Marine Biological Laboratory at Wood's Holl. I would also acknowledge my indebtedness to Prof. S. F. Clarke, of Williams College, and to Mr. W. S. Nickerson, of Harvard, for sending me material.

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ABBREVIATIONS.

<i>a.</i>	Anus.	<i>l.c.</i>	Longitudinal canal.
<i>a.z.</i> , <i>a.z.</i> , ¹ <i>a.z.</i> , ² etc.	Adoral zone.	<i>m.</i>	Membranella.
<i>b.p.</i>	Buccal pouch.	<i>mcn.</i>	Micronucleus.
<i>b.p.m.</i>	Basal plate of membranella.	<i>mgn.</i>	Meganucleus.
<i>c.f.</i>	Connecting filament uniting membranellæ.	<i>mgn.</i> ¹	Anlage of meganucleus.
<i>cl.</i>	Cilium.	<i>mn.</i>	Myoneme.
<i>c.s.</i>	Clear stripe.	<i>o.</i> , <i>o.</i> , ¹ <i>o.</i> , ² etc.	Mouth.
<i>c.v.</i> , <i>c.v.</i> ¹	Contractile vacuole.	<i>o.</i> ¹ <i>inv.</i>	Oral invagination.
<i>d.a.z.</i> ¹	Distal extremity of adoral zone.	<i>p.</i> , <i>p.</i> ¹	Peristome band.
<i>ecp.</i>	Ectoplasm.	<i>pl.</i>	Pellicula.
<i>enp.</i>	Endoplasm.	<i>ps.</i> , <i>ps.</i> ¹	Foot.
<i>ex.p.</i>	Excretory pore of contractile vacuole.	<i>p.z.</i>	Pigmented zone of foot.
<i>f.</i> , <i>f.</i> , ¹ <i>f.</i> ²	Frontal field.	<i>r.b.s.</i>	Right boundary-stripe of ramifying zone.
<i>fc.</i>	Fæcal matter.	<i>r.z.</i> , <i>r.z.</i> ¹	Ramifying zone.
<i>f.v.</i>	Food vacuole.	<i>sh.</i>	Sheath.
<i>g.s.</i>	Granular stripe.	<i>t.f.</i>	Terminal filament of membranella.
<i>l.</i>	Line of fission.	<i>v.</i>	Vacuole.
<i>l.b.s.</i>	Left boundary-stripe of ramifying zone.	<i>vel.</i> , <i>vel.</i> ¹	Velum.

EXPLANATION OF PLATE XXIII.

FIG. 1. *Stentor igneus* var. *nigricans*, var. nov. From Williamstown, Mass. Ventral aspect of a living, semi-contracted specimen, under slight cover-glass pressure. $\times$ ca. 250.

FIG. 2. *Stentor pyriformis*, sp. nov. Ventral aspect of a living, fully-extended specimen (outlined with camera). $\times$ 230.

FIG. 3. *S. pyriformis*. Hardened and stained preparation to show meganuclei. $\times$ 110.

FIG. 4. *S. roeselii*. Living, extended specimen, containing numerous minute algae taken in as food. Dorsal aspect. Zeiss, obj. A. oc. 4.

FIG. 5. Transverse optical section of ectoplasm and pellicula of *S. caeruleus*. The pellicula is adherent only over the myonemes. 1% osmic acid preparation. Zeiss, 4 mm. apoc. obj., oc. 4 = $\times$ 433.

FIG. 6. Part of adoral zone of *S. caeruleus*. Living specimen under strong pressure. Zeiss, 4 mm., apoc. obj., oc. 12 = ca. 650.

FIG. 7. Two adjacent rows of cilia and myonemes, frontal field of *S. caeruleus*. Osmic acid preparation. Zeiss, $\frac{\text{oc. 18}}{\text{obj. 4 mm.}}$ (cam.) = $\times$ 1950.

FIG. 8. Posterior face of transverse section of *S. caeruleus*, taken at time of greatest concentration of meganucleus in fission. Zeiss, $\frac{4}{8 \text{ mm.}}$ = $\times$ 230.

FIG. 9. Stripes of *S. pyriformis*. 1% osmic preparation. Zeiss, $\frac{4}{4 \text{ mm.}}$ = $\times$ 433.

FIG. 10. Myonemes of ramifying zone, *S. caeruleus*. In relaxed condition. Living specimen under strong pressure. Zeiss, $\frac{8}{4 \text{ mm.}}$.

FIG. 11. Myonemes of *S. caeruleus*. From specimen fixed with picro-acetic, stained with borax-carmin. Zeiss, Homog. immers., 2 mm., oc. 2 = $\times$ 428.

FIG. 12. Posterior extremity of *S. caeruleus*. 1% osmic preparation, flattened by cover-glass. $\frac{4}{4 \text{ mm.}}$ = $\times$ 433.

FIG. 13. Longitudinal optical section of new adoral zone of *S. caeruleus*. Corrosive sublimate preparation, $\frac{2}{D}$ = $\times$ 380.

FIG. 14. Foot of *S. caeruleus*, attached to surface film of water. Zeiss, $\frac{12}{16 \text{ mm.}}$.

FIG. 15. Foot of *S. roeselii*, attached in slime. Zeiss, $\frac{4}{D}$.

FIG. 16. Foot of *S. polymorphus*, attached to glass. Zeiss, $\frac{4}{D}$.

FIGS. 17, 18. Abnormal forms of meganucleus, *S. caeruleus*. (17, $\frac{4}{16 \text{ mm.}}$ = $\times$ 112; 18, $\frac{4}{8}$ = $\times$ 230.)

FIG. 19. Two nodes of meganucleus, *S. caeruleus*, with adherent micronuclei (mcn.). The nodes contain vacuolar spaces. Aceto-methyl-green preparation, in H_2O . $\frac{4}{4 \text{ mm.}}$ = $\times$ 433.

FIG. 20. Node of meganucleus, *S. caeruleus*, with several vacuolar spaces of different size, containing each a highly-refractive body. Aceto-methyl-green, glycerin. Zeiss, $\frac{3}{D}$ = $\times$ 550.

FIG. 21. Meganucleus of *S. igneus*, elongated for division. Median optical section, showing chromatic network. Aceto-methyl-green, H_2O . Zeiss, $\frac{4}{D}$ = $\times$ 630.

FIGS. 22-24. Nuclear multiplication in *S. igneus*. Zeiss, $\frac{4}{8}$ = $\times$ 230.

FIG. 25. Meganucleus, *S. igneus*, with adherent micronuclei. Corrosive sublimate, borax-carmin, benzole-balsam. Zeiss, 2 mm. homog. immers., comp. oc. 4 = $\times$ 857.

EXPLANATION OF PLATE XXIV.

All figures on this plate, except Fig. 29, illustrate the fission of *S. caeruleus*.

FIG. 26. Early stage, previous to formation of new mouth, and at beginning of coalescence of meganucleus. Latero-dorsal aspect of extended specimen. From life. Zeiss, $\frac{8}{16}$.

FIG. 27. Later stage. New mouth (σ^1) has formed, and meganucleus (*mgn.*) has divided and begun to elongate. Ventral aspect. The new contractile vacuole, *c. v.*¹, has become abnormally enlarged. From life. Zeiss, $\frac{8}{16}$.

FIG. 28. Same stage as Fig. 27, animal contracted. New adoral zone strongly curved. Blue stripes crenated. Ventro-lateral aspect. From life. Zeiss, $\frac{8}{16}$.

FIG. 29. Newly-formed contractile vacuole of *S. roeselii*. Longitudinal optical section, drawn from living animal. Zeiss, $\frac{1}{8}$.

FIG. 30. Stage of fission a little later than Figs. 27 and 28. The new zone is bent sharply towards the left, at the point where the fission-line would afterwards appear. Animal extended, latero-dorsal aspect. Drawn from life. Zeiss, $\frac{1}{8}$.

FIG. 31. Mid-dorsal portion of semi-contracted specimen, showing fission-line (*l*) running from dorsal border of new zone (*a. z.*¹) around body to right. From life. Zeiss, $\frac{1}{8}$.

FIG. 32. Fission-line at a little later stage than in Fig. 31. It runs above the upper curve of the new zone, forming a conspicuous cleft. Hardened and fully-contracted specimen, dorsal aspect. Zeiss, $\frac{4}{8} = \times 230$.

FIGS. 33, 35-37. Later stages of fission, showing the process of constriction and shaping of frontal field of proximal zoöid. Ventral aspect. Fig. 33 in semi-contracted state; the others fully extended. Drawn from life. Zeiss, $\frac{8}{16}$.

FIG. 34. Five stripes and portion of fission-line, showing that, in contracted state, the stripes form an angle at the constriction-line. Picro-acetic, Delafield's haematoxylin, clove-oil. Zeiss, $\frac{4}{8 \text{ mm.}} = \times 230$.

EXPLANATION OF PLATE XXV.

Regeneration of frontal field and nuclear changes of *S. caeruleus*.

FIG. 38. Specimen in process of regeneration of mouth and frontal field. The buccal portion of adoral zone has already atrophied, and the buccal pouch is much reduced; the meganucleus (*mgn.*) has elongated after condensation. Zeiss, $\frac{8}{16}$.

FIG. 39, A and B. Two successive regenerations of same individual. From life; 39 B one-and-a-half hour later than 39 A. $\frac{2}{8}$.

FIG. 40. Distal individual just after a fission which, in its earlier stages, proceeded like a regeneration. Disorganization of stripes in ramifying zone, due to abnormal mode of fission. From life. Zeiss, $\frac{1}{8}$.

FIG. 41 *a-i*. Series showing changes in living meganucleus from time of greatest condensation to beginning of nodulation. Period of observation, 1.17 P. M. to 2.20 P. M. Zeiss, $\frac{1}{16}$ $\times$ ca. 160.

FIGS. 42, 43. Two consecutive stages in division of meganucleus, after completion of nodulation. In Fig. 43 division has just taken place. From preparations. Zeiss, $\frac{4}{A}$ (*cam.*) $\times$ 150.

FIGS. 44, 45. Two modes of nodulation of meganucleus; 44 somewhat earlier than 45. Zeiss, $\frac{2}{D}$ (*cam.*) $\times$ 380.

FIG. 46 *a-g*. Series showing changes in living meganucleus from division (in condensed stage) to complete nodulation. Zeiss, $\frac{8}{16}$ = ca. $\times$ 100.

FIG. 47 *a-h*. Changes in living meganucleus from beginning of condensation to beginning of renodulation. Zeiss, $\frac{8}{16}$ = ca. $\times$ 100.

FIG. 48. Tangential section of meganucleus in condensed state, with six micronuclei, two of them in mitotic division. Corr. sublimate, picro-carmin. Zeiss, homog. immers. 2 mm. and comp. oc. 4 = $\times$ 857.

EXPLANATION OF PLATE XXVI.

Stentor caeruleus: conjugation, teratology, nutrition, defecation, and merotomy.

FIG. 49. To show line of junction of a conjugated pair. The suture is a dense, refractive plane passing through the whole surface of contact. Zeiss, $\frac{4}{D}$.

FIG. 50. A conjugated pair of very unequal size. Zeiss, $\frac{2}{A} = \times 90$.

FIG. 51. Two separated nodes of meganucleus of conjugated specimen, surrounded and connected by a reticulum of cytoplasm. *In situ*, from living specimen. Zeiss, $\frac{4}{D}$.

FIG. 52. A conjugated pair in Stage H of Maupas. The fragmented meganucleus yet retains its normal stainability. A new meganucleus (*mgn.*¹) is developing in each gamete. In the lower gamete two of the micronuclei are undergoing atrophy within vacuoles. Composite of four transverse sections, 5μ thick. Corrosive sublimate, picro-carmin, xylol balsam. Zeiss, $\frac{4}{8} = \times 240$.

FIGS. 53-59 all studied with Zeiss' Hom. immers., 2 mm. and magnified 1400 diameters.

FIGS. 53, 54. Two micronuclear spindles of different form from same gamete, at "monaster" stage. Corrosive sublimate, picro-carmin, xylol balsam.

FIG. 55. Micronucleus in its normal or resting state. Corrosive sublimate, Czokor's alum cochineal, balsam.

FIG. 56. Micronucleus enlarged at time of conjugation (Stage H, same prep. as Fig. 52).

FIGS. 57, 58. Two stages in enlargement of anlage of new meganucleus. Unstainable. Example in Fig. 58 is accompanied by a micronucleus. Corrosive sublimate, balsam.

FIG. 59. Micronucleus within a digestive vacuole. Unstainable. Corrosive sublimate; Czokor's alum cochineal.

FIG. 60. Node of meganucleus, from an ex-conjugate. (Same gamete as Fig. 57.) Stainability much reduced. Two vesicles of clear karyoplasm under nuclear membrane. Zeiss, Homog. immers., 2 mm. comp. oc. 4 = $\times 950$.

FIGS. 61 A-E. Stages in the formation of a double monster, and its return to the normal condition. All drawn from living animal. Fig. 61 B (dorsal aspect) three hours later than 61 A (ventral aspect). Fig. 61 C (ventral) drawn six hours later than 61 A. Zeiss, $\frac{8}{8}$. Fig. 61 D drawn fifteen hours later than 61 C. Zeiss, $\frac{4}{8}$. Fig. 61 E represents the specimen thirteen hours later than 61 D. Zeiss, $\frac{8}{16}$.

FIG. 62. A *Stentor* feeding upon carmine grains, dorsal aspect. Arrows show the direction taken by the carmine-particles. The stripes are omitted for sake of clearness. Zeiss, $\frac{4}{16}$. From life.

FIG. 63. A specimen of *S. caeruleus* that has fed upon minute green algae (*Scenedesmus*). Defecation taking place at two different points simultaneously, and a few minutes later at the normal place of defecation (*a*). From life. Zeiss, $\frac{8}{16}$.

FIGS. 64 A and B. Posterior merozoön of *S. caeruleus*; A, dorsal, B, ventral aspect; regenerating adoral zone and mouth. A contractile vacuole present, and meganucleus in condensed state (*mgn.*). From life. Zeiss, $\frac{8}{16}$.

